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Effects of low-oxygen atmospheres on impatiens seed germination

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Effects of low-oxygen atmospheres on impatiens seed germination

by

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GENERAL INTRODUCTION

More than half of all bedding plants produced in the United States are grown by using a relatively new growing technique in which seeds are germinated in small, individual cells (plugs) grouped in trays. Subsequently, the seedlings are transplanted to larger containers or the field. The advantages of the plug production system over traditional broadcast seeding in open flats are: 1) better seedling uniformity; 2) less labor; 3) faster production time; 4) adaptability to mechanization; and 5) standardization of production techniques (Hamrick, 1988). It has been estimated that 2.75 billion plugs will be produced in the United States in 1989 (Hamrick, 1988).

A general scheme for the production of plugs follows: plug trays are filled mechanically and conveyed to an automated seeder in which seeds are single, double, and sometimes triple sown. (The seeds also can be treated to enhance seeder accuracy (Hamrick, 1988). For example, marigolds are detailed and/or graphite coated, tomatoes are defuzzed, and begonias are pelleted.) Large seeded species and dark requiring species are covered. The flats are watered and transported to a germination area. Seeds are kept moist by fog or mist systems or may be covered with plastic. This initial period of the production system has been termed "Stage I" and is characterized by high moisture, optimized temperature, and a low light level (Koranski, 1988a). Stage I ends shortly after radicle emergence. Stage II differs form Stage I of production by having a reduced medium-moisture level, a higher light level, and a lower temperature. These conditions promote root growth and cotyledon

expansion. Stages III and IV are refinements of Stage II conditions to promote sturdy, compact seedling growth. The finished plug then is ready to transplant.

Plug trays contain from 128 to 800 individual cells and are 1.5 cm to 4 cm tall. The volume of the individual cells can be as low as 0.5 cm^{-3}. The smallness and shortness of the plug cell in volume and height creates a management problem. Spomer (1975) explained that container media have the seemingly paradoxical property of having too low of a water volume, and too low of a percent airspace. In plug cells, these conditions are even more pronounced (Milks et al., 1989).

The formulation of a medium for use in plug trays is difficult because of the opposing effects of optimizing moisture availability (water volume) and of optimizing aeration (Fonteno, 1988). In general, decreasing the particle size of medium components increases moisture holding characteristics (water volume) with a corresponding medium air space decrease at container capacity (Milks et al., 1989). When component particle size is increased the opposite effect is observed. A medium that drains well will hold little water against gravity, dry out faster, and be difficult to put into a plug tray uniformly because of the larger sized components. Because a medium with a high water holding capacity and low aeration is an easier management problem than is the opposite case, most plug media have a fine, homogeneous component mixture that fills a flat uniformly but holds a maximum amount of water and does not drain well. Milks et al. (1989) has estimated that, even under the best conditions, the air space in the soil medium at container capacity

is probably not greater than 4%.

The limitations imposed by the plug production system result in flower seed germination in high-moisture environments. In addition, the recommendation for germinating most flower seeds is to hold the germination environment at 90 to 100% relative humidity, thus maintaining the high moisture environment (Koranski, 1988b). These conditions increase the chance that seeds are exposed to low-oxygen stress during the germination process. Most discussion among industry representatives has centered around the effects of excess water on germination, rather than too little water, although both problems exist. Armitage (1986) informally surveyed greenhouse operators and reported that seed germination difficulties was one of the problems most cited. The operators who felt seed germination was a problem were primarily plug producers.

Little research has been done to investigate the effects of high moisture content and low aeration on flower seed germination. The reason for this appears to be two-fold. The first, and most likely the primary, reason is that before the plug production system, an individual flower seed was of little value and the fate of individual seeds broadcast in a flat could not be determined easily. The second reason is that flower seeds are small and difficult to study.

The overall objective of this research was to investigate the effects of low-oxygen atmospheres on impatiens seed germination. Within this overall objective, specific objectives were: 1) to identify the sensitivity of impatiens to decreasing oxygen concentrations; 2) to

identify cultivar variability in sensitivity to oxygen; 3) to determine the extent to which seeds placed on top of or buried in the soil medium in a plug tray experience low-oxygen conditions; 4) to determine the physiological response of impatiens seeds to low-oxygen environments; and 5) to relate the observations from laboratory experiments to commercial plug-production systems.

A seedling often cannot survive in low or no oxygen environments, but germinative processes can occur. Shull (1909) reported that high oxygen concentrations are not necessary for germination. Various tolerances to anoxia and hypoxia among species have been reported. Siegel and Rosen (1962) found that celosia, rice, and cucumber could germinate under anaerobic conditions. They also reported that lettuce, onion, and portulaca germinated nearly as well in 2% oxygen as did their air controls. Carrot seeds germinated better in 2% oxygen than they did in air. Morinaga (1926a) tested 78 genera of seeds and found that many could germinate under water. The ability to germinate under water was more generally related to small seed and was not related to phylogeny or to the kind of reserve material in the seed. Oryza sativa (Bertani et al., 1980; Mocquot et al., 1981) and Echinochloa crus-galli (Kennedy et al., 1980; Rumpho and Kennedy, 1981) germinate and survive under water for long periods of time. A number of species germinate as well or better under water as in air (Bewley and Black, 1985; Morinaga, 1926b). Heichel and Day (1972) reported that dark germination and growth in 2%

oxygen was greater in monocots than in dicots as compared to germination and growth in 20.9% oxygen. Ohga (1926) found that lotus seeds had enough oxygen within the seed to allow germination to occur under water or in 100% nitrogen or carbon dioxide gas. Fatty seeds were more sensitive to low oxygen than starchy seeds (Al-Ani et al., 1985). Pisum sativum seeds are intolerant of low-oxygen conditions and they rapidly lose their ability to germinate during prolonged exposure to such conditions (Barclay and Crawford, 1981). Despite the ability of seeds to germinate in low-oxygen environments, most seeds germinate best in oxygen atmospheres approaching that of air, and although germination percentage may not be affected greatly by reduced oxygen, the germination rate almost always is reduced (Al-Ani et al., 1985).

Seed sensitivity to a low oxygen concentration can be caused by several factors. In many cases, the seed coat acts as a barrier to oxygen diffusion to the embryo, or the seedcoat may consume oxygen (Bewley and Black, 1985). A mucilagenous layer forms on the surface of Spinacia oleracea seeds under high moisture conditions, and this prevents oxygen from reaching the embryo (Heydecker et al., 1969). This mucilagenous layer collapses when drier conditions return. Crabb and Kirsop (1969) concluded that a higher oxygen requirement was the difference between water-sensitive and nonwater-sensitive barley. Taylor (1942) surmised that rice could germinate well at oxygen concentrations of less than 1% because of a highly functional fermentation system. Wheat, which germinated poorly under the same conditions, had a poor fermentation system. Barclay and Crawford (1981) reported that anoxic

injury to pea seeds was accelerated as temperature increased between 5 and 25C.

The general response to reduced oxygen levels is an increase in non-oxidative fermentation (Leblova, 1978; Raymond et al., 1985), and many different end products of fermentation have been shown to accumulate. Crawford and Tyler (1969) showed that the roots of flooding-tolerant plants accumulated malate, and flooding-intolerant plants did not accumulate malate. Morohashi and Shimokoriyama (1972a) did not show increased malate in the seeds of Phaseolus mungo, but they found that malate was excreted to the surrounding medium, and therefore they concluded that malate was being synthesized actively early in the germination process. Lactate was found to be high initially in seeds of lettuce and rice, two seeds tolerant to soaking (Crawford, 1977). Soaking-intolerant seeds (pea and maize) produced ethanol as the major end-product of fermentation. None of these seeds accumulated malate. Lactate accumulated in buckwheat seedlings under anaerobiosis (Effer and Ranson, 1967). Buckwheat seedlings also accumulated succinate and ethanol, but malate decreased under anaerobiosis. Several studies have reported small accumulations of amino acids under anaerobic conditions (Effer and Ransom, 1967; Smith and Ap Rees, 1979).

Two lines of experimental evidence have emerged for how plant species tolerate low-oxygen environments. McManmon and Crawford (1971) have theorized that tolerance of low-oxygen conditions is best among plant species that limit the Pasteur effect seen in plants under low oxygen. Tolerant plant species produce less ethanol and instead produce

malate and other organic acids. Pesis and Ng (1984) reported that low-vigor (accelerated aged) Cucumis melo seeds exihibited an apparent Pasteur effect when exposed to 100% nitrogen gas, but high-vigor seeds showed no such effect. In contrast, Bertani et al. (1980) concluded that rice is tolerant of anaerobiosis because it couples a strong alcoholic fermentation to an ability to excrete ethanol to the surrounding medium. Taylor (1942) also attributed a strong alcoholic fermentation to the ability of rice to germinate in 0% oxygen. Smith and Ap Rees (1979) reached a similar conclusion and reported that the marsh plants they studied did not accumulate malate and depended on alcoholic fermentation during anoxia. Other studies have not shown a malate or lactate accumulation (Rumpho and Kennedy, 1981). Most studies with seeds have shown that seeds produce considerably more ethanol than lactate or malate and that during anoxia, ethanol is excreted to the imbibition medium and/or the atmosphere, rather than metabolized (Bertani et al., 1980; Crawford, 1977; Raymond et al., 1985; Rumpho and Kennedy, 1981). Oryza sativa (Bertani et al., 1980) and Echinochloa crus-galli (Rumpho and Kennedy, 1981) excreted 98% and 85%, respectively, of the ethanol produced to the imbibition medium.

Some species exhibit a natural period of anaerobiosis during germination, but this period is short and does not appear to be detrimental to the germination process (Al-Ani et al., 1985; Come and Tissaoui, 1972; Morohashi and Shimokoriyama, 1972b). Moreland et al. (1974) suggested that fermentation, as a whole, may be a survival mechanism whereby the seed withstands unfavorable conditions until more

favorable conditions prevail.

When anaerobic conditions are removed, ethanol and lactate are metabolized rapidly (Cameron and Cossins, 1967; Effer and Ranson, 1967; Leblova et al., 1969). Ethanol and lactate both appear to be metabolized primarily to organic acids of the tricarboxylic acid cycle and acidic amino acids, with a small amount of CO_2 produced as well (Cossins and Beevers, 1963; Cameron and Cossins, 1967; Cossins, 1978). The ultimate end-product of ethanol and lactate metabolism is glutamate and glutamine (Cossins and Beevers, 1963).

Ethanol damages membranes of Nitella (Kiyosawa, 1975) and soybean (Priestley and Leopold, 1980). Isolated hypocotyl cells of Phaseolus vulgaris, placed in 3% ethanol, showed inhibited respiration, and inhibited RNA, protein, and lipid synthesis (DeVilliers et al., 1980). These studies have been done at high exogenous ethanol concentrations, and Jackson et al. (1982) question the role of ethanol in causing injury in low-oxygen environments. Crawford and Zochowski (1984) found that circulating the anaerobic atmosphere gave a 13-fold reduction in the ethanol that accumulated in Cicer arietinum seedlings as compared with the ethanol found in the seedlings in a non-circulating, anaerobic atmosphere, and they concluded that ethanol may have contributed to seedling death under anoxia. Lactate, upon acidification, is highly toxic to cells, but the specific damage to the cell is not reported (Pradet and Bomsel, 1978). The primary defense against lactate accumulation may be a switch to ethanol production (Davies et al., 1974). This switch is triggered by a drop in pH and accumulations of adenosine

triphosphate and pyruvate, and this inhibits lactate dehydrogenase and stimulates pyruvate decarboxylase (PDC). Alcohol dehydrogenase (ADH) activity tends to increase under such conditions (Bertani, et al., 1980), but ADH activity and ethanol production always are not correlated highly (Chang et al., 1982; Wignarajah and Greenway, 1976). PDC is more likely to be the control point of ethanol production (Chang et al., 1983; John and Greenway, 1976).

Explanation of thesis format

This dissertation is arranged in the alternate format consisting of four papers that will be submitted to scientific journals. Paul Karlovich was the principle investigator on all research reported herein, and he is the first author on all four papers. Drs. Gladon and Koranski served as the Major Professors for Paul in his research and are listed as authors on all papers.

SECTION I. GERMINATION OF EIGHT IMPATIENS CULTIVARS UNDER WATER AND ON BLOTTER PAPER

GERMINATION OF EIGHT IMPATIENS CULTIVARS UNDER WATER AND ON BLOTTER PAPER

Paul T. Karlovich, Richard J. Gladon, and David S. Koranski

ABSTRACT

Eight cultivars of Impatiens wallerana Hook. f. from three seed sources were tested on blotter paper and under water to compare germination characteristics. Germination under water reduced germination rate and germination meantime in all cultivars except 'Super Elfin Orchid'. Germination percent after seven days was 15 to 55% lower in five of the eight cultivars when germination took place under water. Results indicate little variability among cultivars that were germinated under ideal conditions (on blotter paper), but much greater variability was found when the seeds were germinated under the severe condition of submergence in water, a condition likely to be encountered by impatiens seeds that are germinated by using current plug-production practices.

INTRODUCTION

Most impatiens seedlings currently are produced by using a system that produces single seedlings in small growing volumes in trays (plugs). For impatiens, these trays usually are 2.5 cm or less in height, and this causes water drainage problems. Milks et al. (1989) has estimated that, under the best conditions, air space at container capacity in the medium of trays of this height is not greater than 4 to 5%. Low-oxygen exposure studies have shown that Impatiens 'Super Elfin Orchid' (SEO) will not germinate in seven days in zero or 1% oxygen, and SEO germinates slowly (as compared to in air) at 3, 5, or 7% oxygen (Karlovich et al., 1988). In contrast, preliminary studies related to this researach showed that SEO seeds germinated as fast under water as they did in air. This is not unusual because many seeds can germinate under water (Bertani et al., 1980; Morinaga, 1926; Rumpho and Kennedy, 1981). Seeds that germinate under water, generally germinate in an oxygen-free atmosphere as well (Rumpho and Kennedy, 1981). However, Morinaga (1926) reported that seeds of several species germinated under water but not in gaseous atmospheres scrubbed of O_2. This study was conducted to compare germination characteristics of eight impatiens cultivars under water and on blotter paper as part of our effort to determine the extent of moisture-induced low-oxygen stress to impatiens seeds germinating in plug trays.

MATERIALS AND METHODS

Eight impatiens cultivars from three seed companies were used for this study: 'Super Elfin Coral' (SEC), 'Super Elfin Orchid' (SEO), and 'Super Elfin Lipstick' (SEL) (Ball Seed Co., West Chicago, IL); 'Rose Star' (RS) (HG German Seeds, Smethport, PA); 'Accent Rose'(AR1 and AR2) (two sister, breeding lines), 'Accent Salmon' (AS), and 'Accent Pink' (AP) (Goldsmith Seeds, Inc., Gilroy CA). Thirty seeds either were submerged in 30 ml of deionized water (>18 MegOhm$\cdot$cm^{-1} resistance) in a 100-ml glass beaker or placed on two layers of Steel Blue Anchor Seed Germination Blotter™ paper (Anchor Paper, St. Paul, MN) saturated with deionized water in 100 × 15 mm plastic petri dishes. Germination counts (radicle emergence) were taken daily for seven days. Percent germination, germination rate (Timson, 1965), coefficient of uniformity of germination (CUG) (Bewley and Black, 1985), and germination meantime (Bewley and Black, 1985) were calculated. Tests were conducted on a laboratory bench at 23C under existing fluorescent lighting (4 to 7 μmol$\cdot$s$^{-1}\cdot$m^{-2} inside the mason jar) for 8 to 10 hr daily. The experiment was analyzed as a completely randomized design replicated six times over time. The average seed weight by cultivar was calculated by using four replications of 200 seeds each. However, Duncan's Multiple Range test was performed only on SEL, SEO, SEC, and RS because of insufficient seed numbers for the other cultivars.

RESULTS

Significant differences were found for germination rate, germination percent, germination meantime, and CUG for cultivars, treatments, and their interaction (Table 1). Treatment was the most important effect, and the cultivar and cultivar-treatment interaction effects were approximately equal. The germination rate, germination percent, germination meantime, and CUG by cultivar and by treatment are listed (Table 2).

Percent germination for each treatment-cultivar combination displayed an interaction effect (Figure 1). On blotter paper, germination percent was 90 or greater for all cultivars, and no significant differences occurred among cultivars. Submersion in water did not affect the germination percent of any of the cultivars in the Super Elfin series, and all cultivars of this series averaged greater than 95% germination. However, submersion in water reduced the germination percents of the other cultivars to 82 (RS) to 40% (AR2), and the germination percent of these cultivars was different from their germination percent on blotter paper.

Germination percent data do not give an adequate representation of the effect of submersion in water on the germination process. Germination rates were different among cultivars germinated on blotter paper (ranging from 478 for RS to 404 for AR2), but the largest differences occurred between treatments (Figure 2). Within each cultivar, treatment differences were significant except for SEO. As with percent germination, the Super Elfin series was affected less than was RS and the Accent series (AR1, AR2, AS, and AP). The germination rate of AR2 was affected the most by submersion in water.

Within each cultivar, differences between treatments in germination meantime were significant for all cultivars except SEO (Figure 3). On blotter paper, the germination meantime ranged from 3.1 (RS) to 3.9 days (SEC), while germination meantime of submerged seeds ranged from 3.3 (SEO) to 5.8 (AR2) days. As it was observed for germination rate (Figure 1), among cultivars some of the blotter paper germination meantimes are different, but the greatest differences occurred between treatments.

The CUG is a unitless number that indicates seed germination synchronicity around the germination meantime. The CUG was lower when the seeds were submerged as compared with blotter paper, but only SEL and RS had treatment differences (Figure 4). On blotter paper the CUG for RS was larger than the CUG for any other cultivar, but the CUG of RS was reduced to a value similar to the values for the other cultivars when RS was submerged.

The average seed weight for the eight impatiens cultivars tested ranged from 0.52 to 0.66 $mg \cdot seed^{-1}$ (Table 3). SEO and SEC were the smallest seeds (0.52 and 0.53 $mg \cdot seed^{-1}$, respectively), and they were significantly smaller than RS and SEL (0.59 and 0.60 $mg \cdot seed^{-1}$, respectively).

DISCUSSION

The design of a plug tray creates conditions in the soil medium that are characterized by a high percent water content and a low percent airspace at container capacity (Milks et al., 1989). Seeds may be required to germinate under water in a plug tray, particularly if the seeds are buried in the soil medium. The results of this study indicate that impatiens seed cultivars have similar germination characteristics when they are germinated under ideal conditions (on blotter paper), but under the severe condition of being submerged in water, there is a range of responses, all of which are worse than the results obtained on blotter paper. The sole exception to this statement is the cultivar SEO.

SEO germinated equally well on blotter paper and under water. Morinaga (1926) found no phylogenetic relationship in the ability of a seed to germinate under water, but he concluded that smaller seeds germinated better under water. SEO was the smallest seed, but SEC, which was almost the same size did not germinate as fast under water. At the other extreme, AS was the largest seed, but it did not perform as poorly as AR2. Although germination tended to be poorer under water as the seed size increases, the results did not implicate clearly that seed size alone caused these differences.

The ability to germinate under water may be an advantage for impatiens seeds germinated in plug trays. Two cultivars in this study, SEO and SEL, displayed an ability to germinate to a high percent and at a relatively faster rate under submergence conditions than the other cultivars tested (Figures 1 and 2). The reason for this ability to germinate so well under

water would be a good subject of future research. The results of this study are at odds with research we conducted showing that SEO impatiens seeds would not germinate in 0% and 1% oxygen atmospheres created with nitrogen and oxygen gases, and this discrepancy is yet to be explained (Karlovich et al., 1988). Morinaga (1926) reported similar results with several species.

It is interesting that AR1 and AR2 had different germination characteristics (Figures 1-4). These two sister, breeding lines essentially were identical when germinated on blotter paper, but the stress of submergence resulted in significant differences between their germination characteristics. AR1 represents an "improved" version of AR2; both cultivars have the same female parent, but they have different pollen parents (personal communication, Mike Capp, Goldsmith Seeds, Inc., Gilroy, CA). In respect to the response to submergence, AR1 is an improvement over AR2. It may be that a simple, rapid germination test in which the seeds were submerged would prove useful for the assessment of improved impatiens seed vigor.

An informal survey of the greenhouse industry by Armitage (1986) reported that seed germination was one of five common production problems. The seed germination problem was cited almost exclusively by plug growers. Our results lead us to conclude that cultivar to cultivar variability under stress conditions may be one reason for this problem. Very little research has been done to identify sources of variation among impatiens cultivars, and even less work has been done on lot to lot variation within cultivars. This study was conducted on one lot of each of the impatiens cultivars

listed, and it would be premature to conclude that other lots of the same cultivar would perform in the same manner as reported here. The fact that some cultivars germinated well under water should not be taken to mean that germination under water is a viable option for the germination of seeds of these cultivars. All cultivars had slow subsequent growth when submerged in water during germination. The differences noted in this research have not yet been shown to translate to differences in commercial plug-production systems. Future research should be directed toward: 1) the nature of the ability of impatiens seeds to germinate under water while not under nitrogen atmosphere, and 2) the relationship of germination characteristics among cultivars to seedling plug performance.

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Table 1. Mean squares from ANOVA for germination rate, germination percent, germination meantime (GMT), and coefficient of uniformity of germination (CUG)

Source	df	Germination		GMT	
		Rate	Percent	(Days)	CUG
Cultivar (C)	7	44,282 ***	1031 ***	2.74 ***	33.0 ***
Treatment (T)	1	640,158 ***	5766 ***	40.32 ***	285.1 ***
Time	5	3,673 NS	100 NS	0.68 ***	8.2 NS
C × T	7	35,430 ***	1117 ***	1.38 ***	37.5 ***
Error	75	1774	66	0.11	9.5

NS,***Nonsignificant or significant at 0.1% level.

Table 2. Cultivar and treatment effects on germination percent, germination rate, germination meantime (GMT), and coefficient of uniformity of germination (CUG)

	Germination		GMT	
	Percent	Rate	(Days)	CUG
Cultivar				
Super Elfin Orchid	93	438	3.3	3.0
Super Elfin Lipstick	97	423	3.6	3.5
Rose Star	90	368	4.0	6.7
Super Elfin Coral	96	355	4.3	2.8
Accent Salmon	87	334	4.3	2.2
Accent Pink	88	326	4.4	1.3
Accent Rose 1	89	320	4.5	2.1
Accent Rose 2	67	246	4.8	2.3
$LSD_{0.05}$	7	34	0.3	2.5
Treatment				
Blotter Paper	96	433	3.5	4.7
Submerged	81	270	4.8	1.3
Significance	***	***	***	***

***Significant at 0.1%.

Table 3. Mean seed weights and standard deviations for eight impatiens cultivars

Cultivar	Mean Seed Weight (mg)	SD
SEL	0.60b[a]	1.65
SEO	0.52a	4.87
SEC	0.53a	4.42
RS	0.59b	0.56
AR1	0.60	-
AS	0.66	-
AP	0.60	-
AR2	0.63	-

[a]Mean separation by Duncan's multiple range test, 5% level. Cultivars with no letter are average seed weights based on less than 200 total seeds, and they were not included in the analysis.

Figure 1. Germination percent for eight impatiens cultivars germinated on blotter paper or submerged in water. Cultivar abbreviations are 'Super Elfin Lipstick' (SEL), 'Super Elfin Orchid' (SEO), 'Super Elfin Coral' (SEC), 'Rose Star' (RS), 'Accent Rose' 1 (AR1), 'Accent Salmon' (AS), 'Accent Pink' (AP), and 'Accent Rose' 2 (AR2)

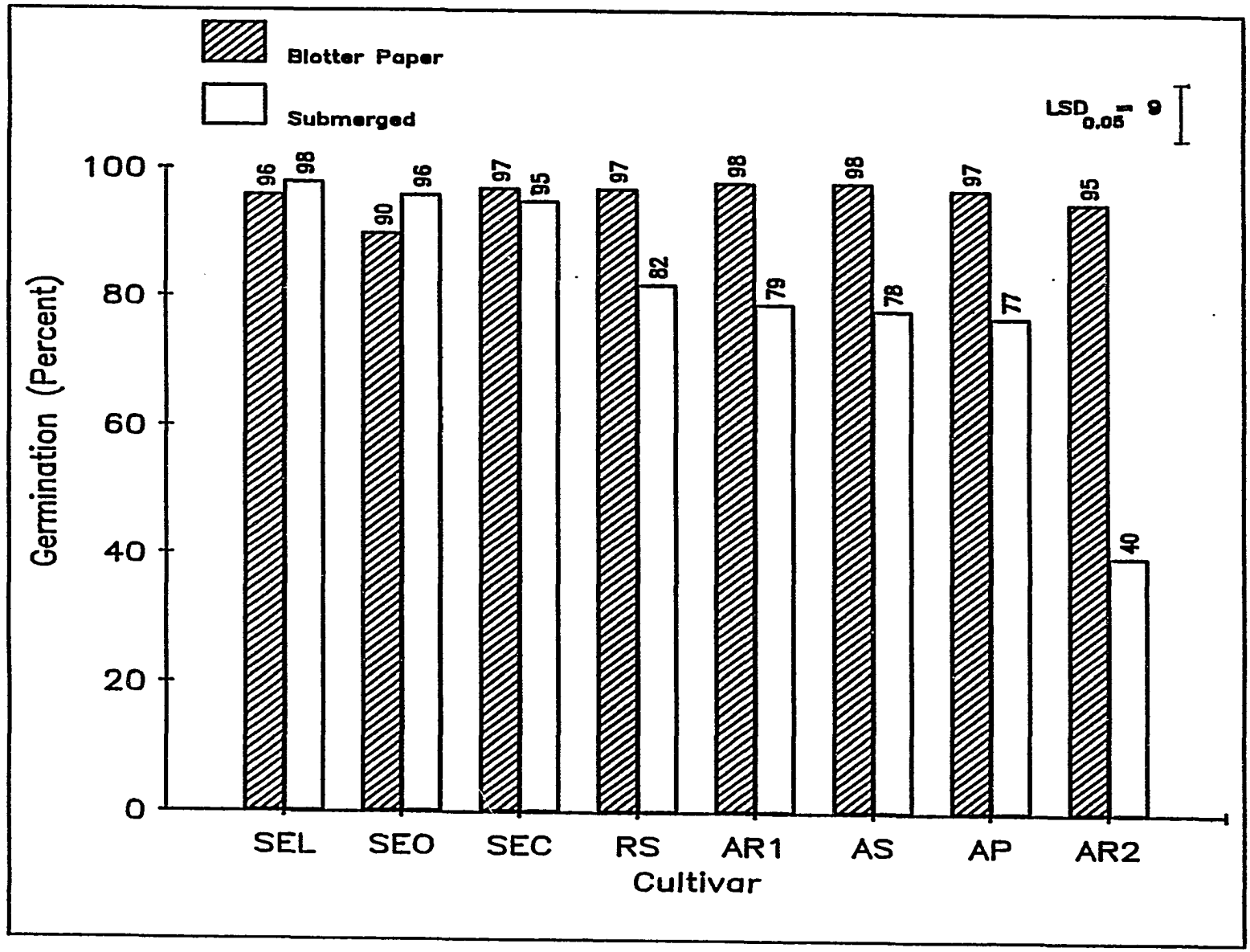

Blotter Paper
Submerged
LSD0.05 = 9
Germination (Percent)
100
80
60
40
20
0
96
98
90
96
97
95
97
82
98
79
98
78
97
77
95
40
SEL
SEO
SEC
RS
AR1
AS
AP
AR2
Cultivar

Figure 2. Germination rate for eight impatiens cultivars germinated on blotter paper or submerged in water. Cultivar abbreviations are 'Super Elfin Lipstick' (SEL), 'Super Elfin Orchid' (SEO), 'Super Elfin Coral' (SEC), 'Rose Star' (RS), 'Accent Rose' 1 (AR1), 'Accent Salmon' (AS), 'Accent Pink' (AP), and 'Accent Rose' 2 (AR2)

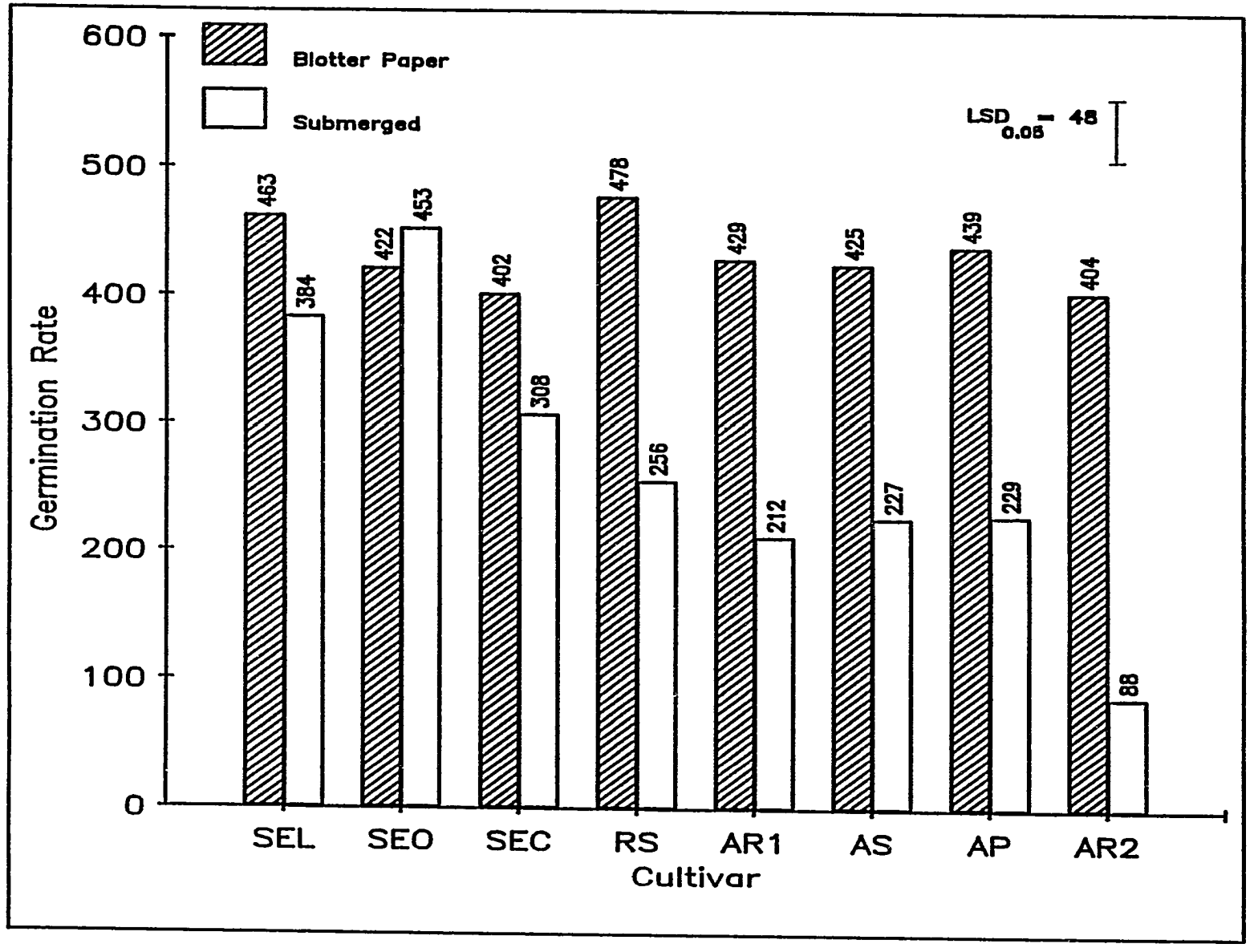

Blotter Paper
Submerged
LSD 0.05 = 48
Germination Rate
600
500
400
300
200
100
0
463
384
422
453
402
308
478
256
429
212
425
227
439
229
404
88
SEL
SEO
SEC
RS
AR1
AS
AP
AR2
Cultivar

Figure 3. Germination meantime for eight impatiens cultivars germinated on blotter paper or submerged in water. Cultivar abbreviations are 'Super Elfin Lipstick' (SEL), 'Super Elfin Orchid' (SEO), 'Super Elfin Coral' (SEC), 'Rose Star' (RS), 'Accent Rose' 1 (AR1), 'Accent Salmon' (AS), 'Accent Pink' (AP), and 'Accent Rose' 2 (AR2)

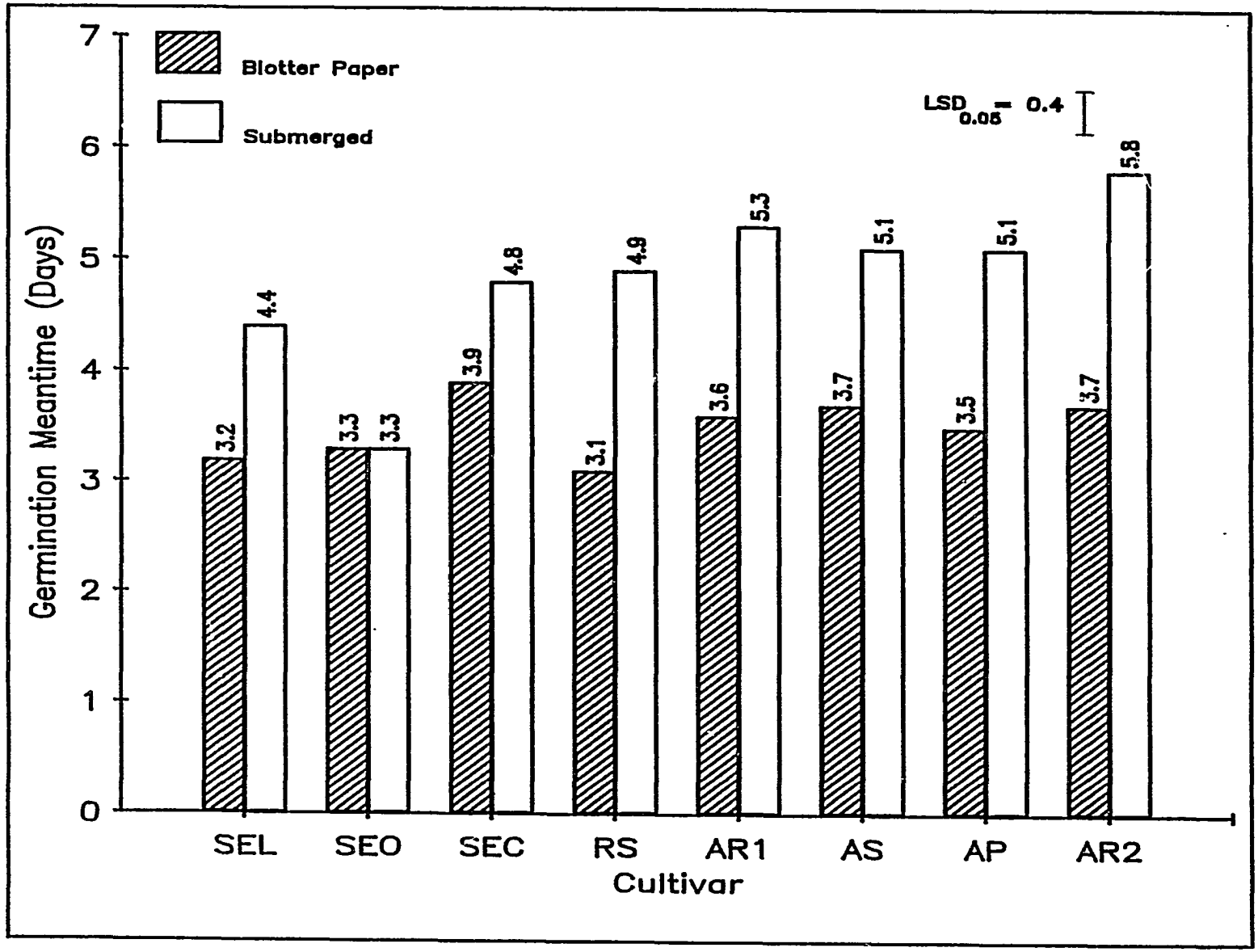
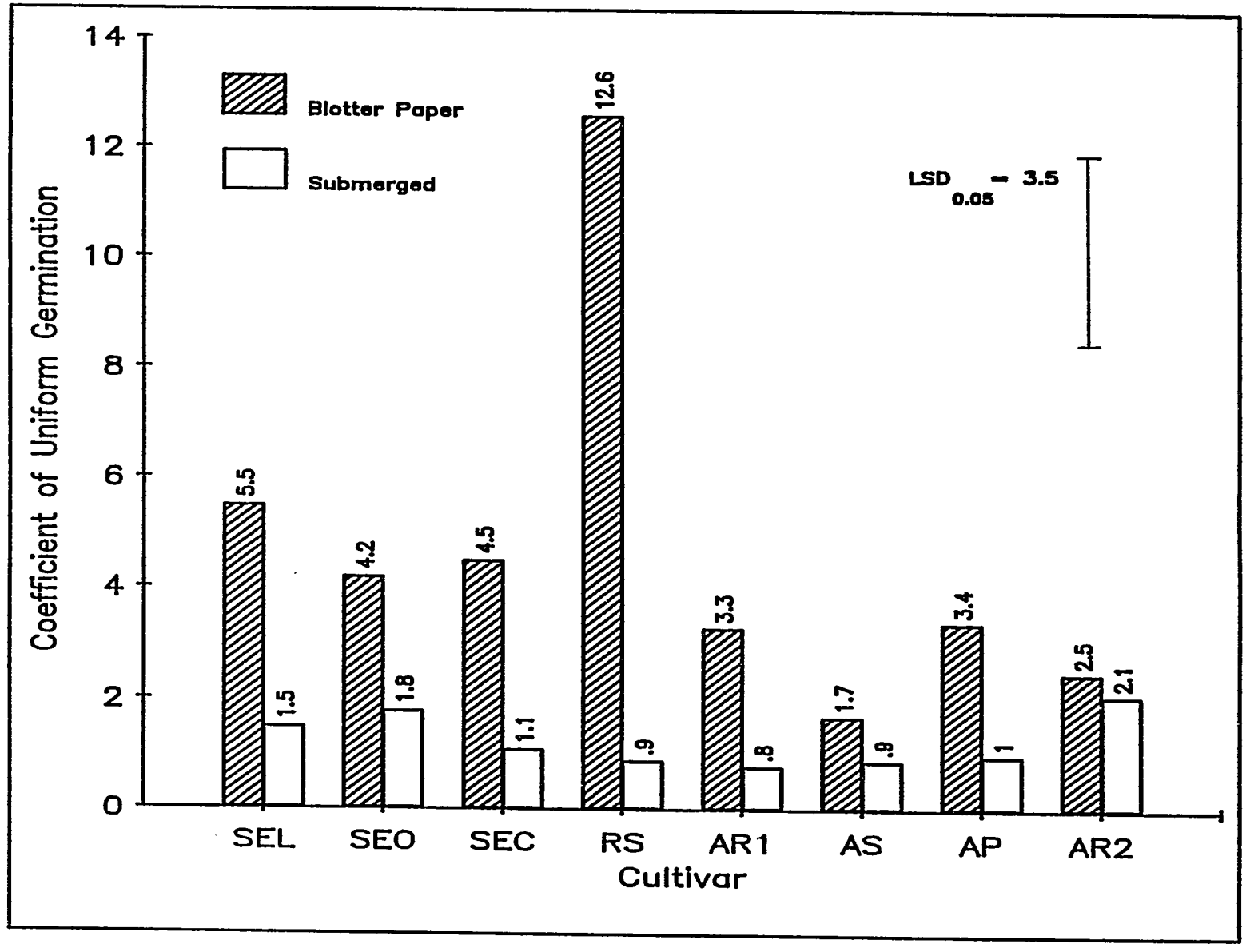

Blotter Paper
Submerged
LSD 0.05 = 0.4
Germination Meantime (Days)
0
1
2
3
4
5
6
7
3.2
4.4
3.3
3.3
3.9
4.8
3.1
4.9
3.6
5.3
3.7
5.1
3.5
5.1
3.7
5.8
SEL
SEO
SEC
RS
AR1
AS
AP
AR2
Cultivar

Figure 4. Coefficient of uniformity of germination (CUG) for eight impatiens cultivars germinated on blotter paper or submerged in water. Cultivar abbreviations are 'Super Elfin Lipstick' (SEL), 'Super Elfin Orchid' (SEO), 'Super Elfin Coral' (SEC), 'Rose Star' (RS), 'Accent Rose' 1 (AR1), 'Accent Salmon' (AS), 'Accent Pink' (AP), and 'Accent Rose' 2 (AR2)


Coefficient of Uniform Germination
14
12
10
8
6
4
2
0
Blotter Paper
Submerged
LSD 0.05 = 3.5
5.5
1.5
4.2
1.8
4.5
1.1
12.6
.9
3.3
.8
1.7
.9
3.4
1
2.5
2.1
SEL
SEO
SEC
RS
AR1
AS
AP
AR2
Cultivar

SECTION II. OXYGEN CONCENTRATION EFFECTS ON IMPATIENS SEED GERMINATION

OXYGEN CONCENTRATION EFFECTS ON IMPATIENS SEED GERMINATION

Paul T. Karlovich, Richard J. Gladon, and David S. Koranski

ABSTRACT

Impatiens wallerana Hook. f. seeds of the cultivars 'Rose Star', 'Super Elfin Lipstick', and 'Super Elfin Orchid' were exposed either to oxygen concentrations of 0, 3, 7, 10, 13, or 20% for seven days continuously, or to split oxygen regimes of one day at 7% oxygen followed by six days at 20% oxygen or one day at 20% oxygen followed by six days at 7% oxygen. Impatiens seeds were not able to germinate in seven days at 0% oxygen, and essentially no germination occurred in 3% oxygen. From 7% to 20% oxygen, a linear increase in percentage germination was found. The germination rate and germination meantime of the three cultivars were curvilinear in response to oxygen concentration. 'Rose Star' germination was curtailed more at 7% and 10% oxygen than was 'Super Elfin Lipstick' and 'Super Elfin Orchid'. Linear declines in the coefficient of uniformity of germination were found for all cultivars as the oxygen concentration decreased, and 'Rose Star' germination uniformity was affected the most. These results indicate that high oxygen concentrations are best for optimum germination of impatiens, and the results are contradictory to previous work that showed that impatiens seeds can germinate when submerged in water.

INTRODUCTION

A seedling often cannot survive in environments that consist of low or no oxygen, but germinative processes can occur. Shull (1909) reported that high oxygen concentrations are not necessary for germination, and various tolerances to anoxia or hypoxia among species have been reported (Al-Ani et al., 1985; Morinaga, 1926a,b). Oryza sativa (Bertani et al., 1980; Mocquot et al., 1981) and Echinochloa crus-galli (Kennedy et al., 1980; Rumpho and Kennedy, 1981) germinate and survive under water for long periods of time. Ohga (1926) found that lotus seeds had enough oxygen within the seed to allow germination to occur under water, in 100% nitrogen, or in 100% carbon dioxide gas. Other seeds (e.g., Pisum sativum) are intolerant of low-oxygen conditions, and they rapidly lose their ability to germinate during prolonged exposure to low-oxygen conditions (Barclay and Crawford, 1981). Despite the ability of some seeds to germinate in low-oxygen environments, most seeds germinate best in oxygen atmospheres approaching that of air, and although germination percentage may not be affected greatly by reduced oxygen, the germination rate is reduced (Al-Ani et al., 1985).

Armitage (1986) informally surveyed greenhouse operators and reported that seed germination difficulties was one of the problems most cited. The operators who felt seed germination was a problem primarily were plug producers. Plug cells have a small volume and short height. Spomer (1975) explained that container media have the seemingly paradoxical property of having too low a water volume, and too low a percent airspace. In plug cells, these conditions are even more pronounced

(Milks et al., 1989). Formulation of a medium for use in plug trays is difficult because of the opposing effects of optimizing moisture availability (water volume) and of optimizing aeration (Fonteno, 1988). Most plug media have a fine, homogeneous component mixture that fills a flat uniformly (a major difficulty with coarse media), but holds a maximum amount of water and does not drain well.

These limitations, imposed by the plug-production system, result in flower seed germination in high-moisture environments. In addition, the recommendation for germinating most flower seeds is to hold the germination environment at 90 to 100% relative humidity, and this maintains the high-moisture environment (Koranski, 1988). These conditions increase the chance that seeds are exposed to low-oxygen stress during the germination process. This study observed the effects of various oxygen concentrations and combinations of concentrations on impatiens seed germination as part of our efforts to determine the extent to which impatiens seeds are oxygen-stressed during germination in plug trays.

MATERIALS AND METHODS

Seeds of impatiens cultivars 'Rose Star' (H.G. German Seeds, Smethport, PA), 'Super Elfin Lipstick', and 'Super Elfin Orchid' (Ball Seed Co., West Chicago, IL) were exposed either to oxygen concentrations of 0, 3, 7, 10, 13, or 20% for seven days continuously, or to split oxygen regimes of one day at 7% oxygen followed by six days at 20% oxygen, or one day at 20% oxygen followed by six days at 7% oxygen. Fifty seeds were placed on two layers of water-saturated, Steel Blue Anchor Seed Germination Blotter™ (Anchor Paper, St. Paul, MN) in wide-mouth, 473 ml mason jars. The desired oxygen concentrations were achieved by using nitrogen and oxygen compressed gases and a capillary-tube mixing system previously described by Morris (1969) and modified by Ahmad (1985). Oxygen concentrations were verified by using a Varian Model 3760 gas chromatograph and appropriate standards. Conditions for use of the gas chromatograph were described previously (Sinska and Gladon, 1984). The gas flow through each mason jar was approximately 275 $ml \cdot hr^{-1}$.

Once the jars were sealed, it took a maximum of eight hours for all treatments to reach the desired oxygen concentration. Germination was counted at radicle emergence (Bewley and Black, 1985). Daily germination counts were taken, and in addition to percentage germination, germination rate (Timson, 1965), germination meantime, and coefficient of uniformity of germination (CUG) were calculated (Bewley and Black, 1985). The experiment was conducted in a growth chamber at 25C under continuous cool white fluorescent lighting at 16 to 21 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon

flux inside the mason jar. The treatment design was a three by eight factorial, and the experimental design was a randomized complete block with two blocks. The experiment was replicated three times over time. Analysis of variance and linear and quadratic trend analysis were done on the 7, 10, 13, and 20% oxygen continuous exposure treatments. The 0% and 3% oxygen treaments were excluded because these treatments had not germinated in seven days. T-tests were used to compare the split oxygen concentration treatments to continuous exposure treatments of interest.

RESULTS

Highly significant cultivar and treatment differences were found for percentage germination, germination rate, germination meantime, and CUG, and the cultivar and treatment sources of variation accounted for most of the experimental variability (Table 1). The cultivar by treatment interaction also was highly significant for all variables except for the germination percent.

All variables had highly significant linear responses to oxygen concentration, and with the exception of germination percentage, the responses were different between cultivars (C×T) (Table 1). The percentage germination of the three cultivars had a positive, linear response to oxygen concentration (Figure 1). The CUG of the cultivars also had a positive, linear response to oxygen, but the cultivars responded differently (Table 1). The CUG of 'Rose Star' had a sharp, linear decline in response to decreasing oxygen concentration (Figure 2). SEL and SEO also showed a linear decline in CUG as the oxygen concentration declined, but the magnitude of the decline was not nearly as great as for 'Rose Star'. For germination rate and germination meantime, the response to treatment, although primarily linear, showed a smaller quadratic trend (Table 1). The quadratic effect for these variables also was found to be cultivar specific (C×T). A highly significant lack of fit was found for germination rate and germination meantime treatment comparisons.

Of the three cultivars, SEO had the lowest germination percentage, germination rate, and CUG, and the highest germination meantime (Table

2). SEL had the fastest germination rate and germination meantime. 'Rose Star' had the highest CUG. SEL and SEO germination rate and germination meantime responded in similar manner to the oxygen concentration (Figures 3 and 4, respectively). 'Rose Star' responded similarly to SEL and SEO at higher oxygen concentration (13 to 20%), but 'Rose Star' was affected more by lower oxygen concentrations. Seeds that had not germinated in the 7 days of the experiment were able to germinate after being moved to an ambient oxygen environment (data not presented). The final percentage germination was above 90% for seeds from all treatments except for seeds from the continuous 0% oxygen treatment. Seeds from this treatment had a lower final germination percent (46, 73, and 78% for RS, SEL, and SEO, respectively).

Exposure for one day to 7% oxygen followed by six days exposed to 20% oxygen and one day exposure to 20% oxygen followed by six days exposed to 7% oxygen each were compared to the two most closely related continuous oxygen exposure treatments (Table 3). In each case, the response to the two split-oxygen treatments was intermediate between the two nearest continuous exposure treatments for germination rate, germination meantime, and CUG.

DISCUSSION

Impatiens seeds exposed to progressively lower oxygen concentrations showed a linear decline in germination percentage after seven days. The rate and meantime of germination were affected more than the percentage germination. Impatiens seed germination was affected even at the relatively high oxygen levels of 10 and 13%. Impatiens is a fatty seed (see Appendix B), and fatty seeds are more sensitive to oxygen concentration than are starchy seeds, probably because the conversion of fat to sugar begins with the oxygen-requiring β-oxidation pathway (Al-Ani et al., 1985). Impatiens also have a mucilagenous layer inside the seedcoat, and this also could act as a barrier to oxygen and further exacerbate the effects of a reduced oxygen concentration (Atwater, 1980).

Exposure of seeds to one day of 20% or 7% oxygen before moving to 7% or 20% oxygen, respectively, showed an intermediate response to the continuous exposure treatments closest to these treatments. These results suggest that even relatively short durations at low or high oxygen concentrations will affect the germination process.

RS was more sensitive to low-oxygen conditions than SEL and SEO. This sensitivity was particularly evident in the very rapid drop in the CUG as the oxygen concentration decreased, and it also is seen in the relative changes in the germination rate and germination meantime (Figures 2,3, and 4). At 0 and 3% oxygen, none of the cultivars germinated in seven days (2% of the SEO seeds germinated in 3% oxygen). In another study (Karlovich et al., 1988), SEO germinated better at 3% oxygen than in this study. It is possible that seed age or slightly

different experimental conditions affected the results. Regardless of the reason for this, with both experiments the 3% oxygen treatment had a large effect on SEO germination.

The poor general response of SEO to this experimental system is of interest. We noticed that SEO germination percentage was low even at 20% oxygen compared with germination tests done on blotter paper under a static ambient atmosphere. The reason for these results are not certain. SEO seeds that did not germinate to the expected potential for the given oxygen treatment (as compared to the other two cultivars) readily germinated after the experiment when they were placed on blotter paper with no air flow. All treatments except 0% oxygen ultimately achieved greater than 90% germination. A possible explanation for the low germination percentage of SEO is that the air flow through the system was fast enough to disrupt the germination of some SEO seeds. SEL and RS germination was not affected by this system. A static, 20% oxygen treatment showed similar germination percent and rate for SEL and RS as in the dynamic 20% oxygen treatment, but SEO germinated at a rate and percentage as in previous tests on blotter paper (data not presented).

SEO has the ability to germinate under water as fast and to the same percentage as seeds tested on blotter paper (see Section I). This data, taken with the hypothesis that SEO may be susceptible to low air-flow rates, lead us to another hypothesis that a seed coat difference may allow SEO to germinate under water by excreting metabolites inhibitory to the germination process. The metabolite or metabolites may be excreted fast enough to avoid the germination delay seen with other submerged

impatiens cultivars. Ethanol is a metabolite of anaerobic respiration that commonly accumulates and is excreted readily from some seed species (Bertani et al., 1980; Kennedy et al., 1980). If the seed coat is different in a way that allows faster excretion from submerged seeds, then we can envision that such seeds might be more susceptible to drying than other impatiens cultivars. More research is needed to verify this hypothesis.

The results of this research indicate that impatiens seeds are sensitive to oxygen concentration. Twenty percent oxygen resulted in the fastest germination. This implies that for seeds germinated in plug-production systems, care must be exercised to insure that seeds are not exposed to reduced oxygen concentrations. However, these results are complicated by the fact that impatiens seeds will germinate when submerged in water. Although the medium in the plug cell has a low oxygen concentration when saturated, the contradictory results of submergence in water versus in gaseous low-oxygen do not allow us to predict the extent to which impatiens seeds may be oxygen stressed when germinated on the surface of the medium.

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Table 1. Mean squares for treatment main effects and treatment by cultivar interaction partitioned into linear and quadratic components for germination percentage, rate, and meantime (GMT), and coefficient of uniformity of germination (CUG)

Source	Germination (%)	Germination Rate	GMT (Days)	CUG
Cultivar (C)	1851 ***	79,819 ***	1.76 ***	27.8 ***
Treatment (T)	215 ***	109,400 ***	9.64 ***	26.4 ***
Block	18 NS	583 NS	0.12 *	2.8 *
C × T	27 NS	4,440 ***	0.31 ***	10.1 ***
Error	31	775	0.04	1.1
T				
L	488 ***	239,724 ***	20.34 ***	76.0 ***
Q	117 NS	78,948 ***	7.77 ***	3.2 NS
Lack of fit	39 NS	9,530 ***	0.81 ***	0.0 NS
C × T				
L	7 NS	6,789 ***	0.77 ***	30.6 ***
Q	69 NS	6,316 ***	0.17 **	2.0 NS
Lack of fit	4 NS	217 NS	0.00 NS	0.0 NS

NS,*,**,*** t-tests nonsignificant or significant at 5%, 1%, or 0.1% level, respectively.

Table 2. Cultivar and treatment effects on germination percentage, germination rate, germination meantime (GMT), and the coefficient of uniformity of germination (CUG)

Source	Germination (%)	Germination Rate	GMT (Days)	CUG
Cultivar				
Super Elfin Orchid	79	300	4.2	0.9
Rose Star	94	385	3.9	2.8
Super Elfin Lipstick	94	408	3.7	1.9
$LSD_{0.05}$	4	19	0.1	0.7
Treatment				
0	0	0	-	-
3	0	0	-	-
7	84	252	5.0	1.0
10	89	374	3.8	1.3
13	90	406	3.5	1.7
20	92	436	3.3	3.7

Table 3. T-tests that compare one day at 7% O_2 followed by six days at 20% O_2 (7→20) with continuous exposure to 20 or 13% O_2 and one day at 20% O_2 followed by six days at 7% O_2 (20→7) with continuous exposure to 7 or 10% O_2 for percentage germination, germination rate, germination meantime (GMT), and coefficient of uniformity of germination (CUG)

Treatment Comparison	Germination Percentage	Germination Rate	GMT (Days)	CUG
13 vs	90	406	3.5	1.7
7→20	93	410	3.6	2.6
Significance	NS	NS	NS	NS
20 vs	92	436	3.3	3.7
7→20	93	410	3.6	2.6
Significance	NS	NS	*	NS
7 vs	84	252	5.0	1.0
20→7	86	297	4.6	0.8
Significance	NS	*	**	NS
10 vs	89	374	3.8	1.3
20→7	86	297	4.6	0.8
Significance	NS	***	***	**

NS,*,**,*** t-tests nonsignificant or significant at 5%, 1%, or 0.1% level, respectively.

Figure 1. Linear regression of the effect of oxygen concentration on the day-seven germination percentage of impatiens seeds. Each symbol represents the mean of 18 observations

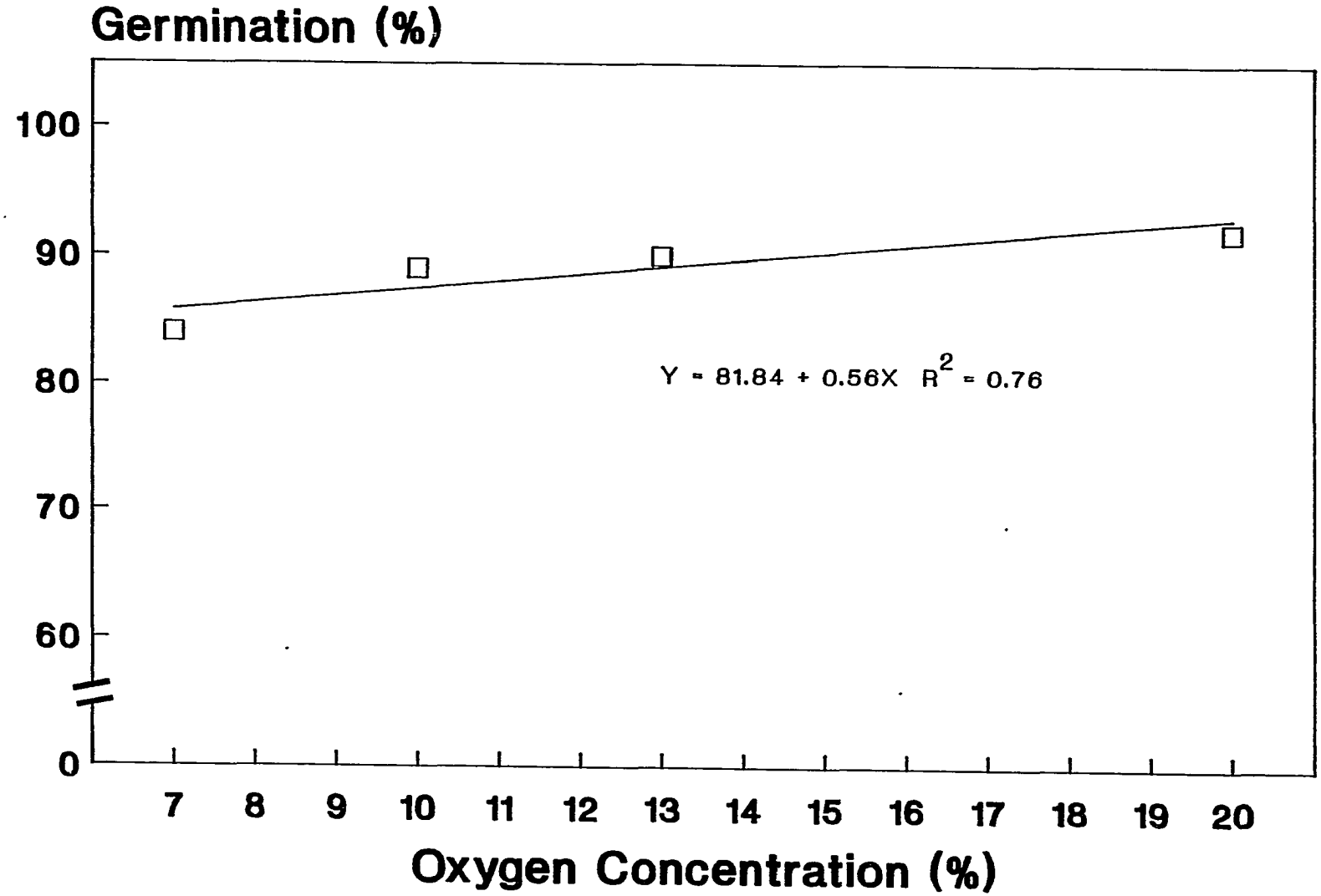

Germination (%)
100
90
80
70
60
0
Y = 81.84 + 0.56X R2 = 0.76
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Oxygen Concentration (%)

Figure 2. Regression lines showing the effect of oxygen concentration on the coefficient of uniformity of germination of seeds of the impatiens cultivars 'Rose Star, 'Super Elfin (SE) Lipstick', and 'SE Orchid'. Each symbol represents the mean of 6 observations

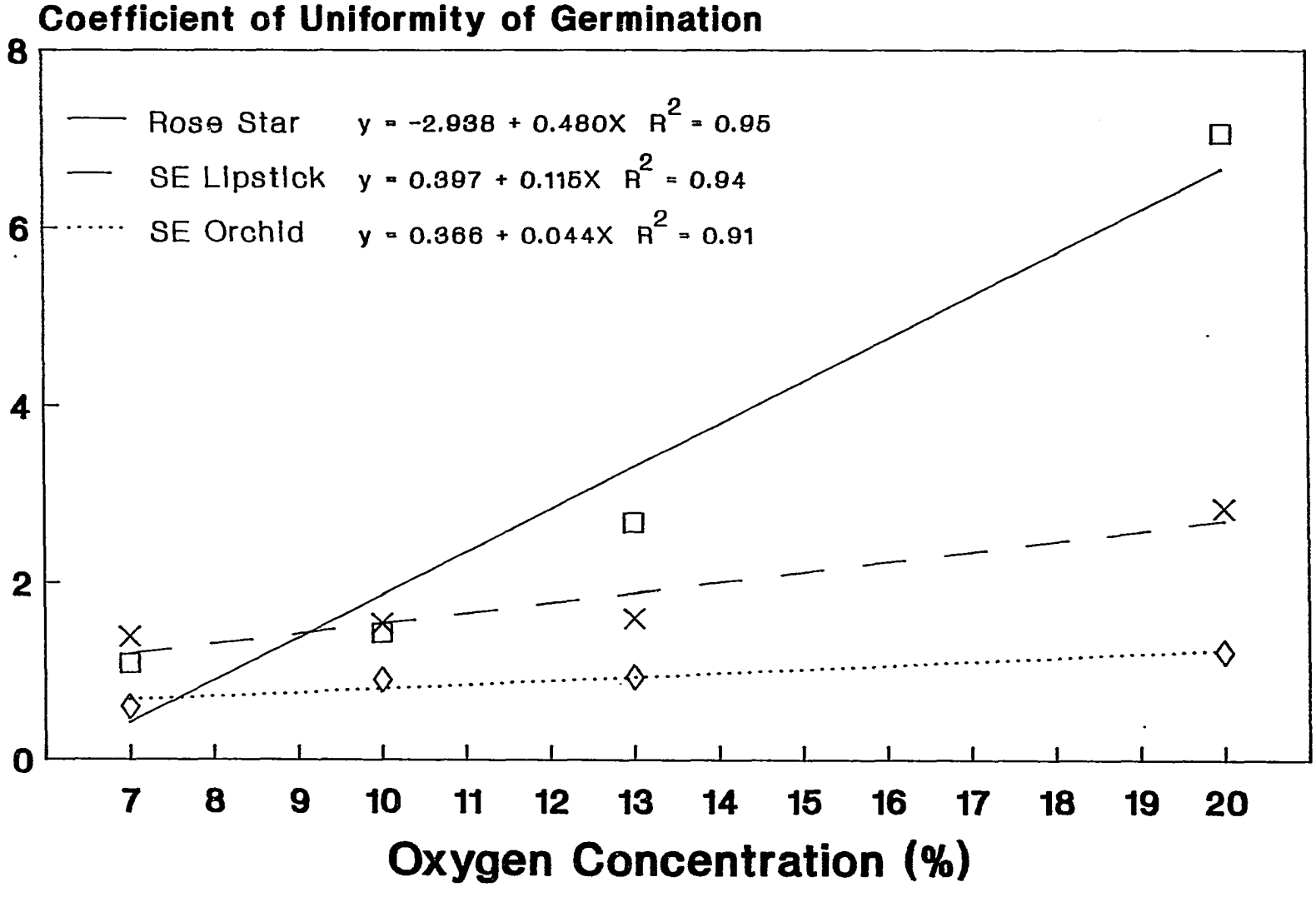

Coefficient of Uniformity of Germination
Rose Star y = -2.938 + 0.480X R2 = 0.95
SE Lipstick y = 0.397 + 0.115X R2 = 0.94
SE Orchid y = 0.366 + 0.044X R2 = 0.91
0
2
4
6
8
7 8 9 10 11 12 13 14 15 16 17 18 19 20
Oxygen Concentration (%)

Figure 3. Regression lines showing the effect of oxygen concentration on the germination rate of seeds of the impatiens cultivars 'Rose Star', 'Super Elfin (SE) Lipstick', and 'SE Orchid'. Each symbol represents the mean of 6 observations

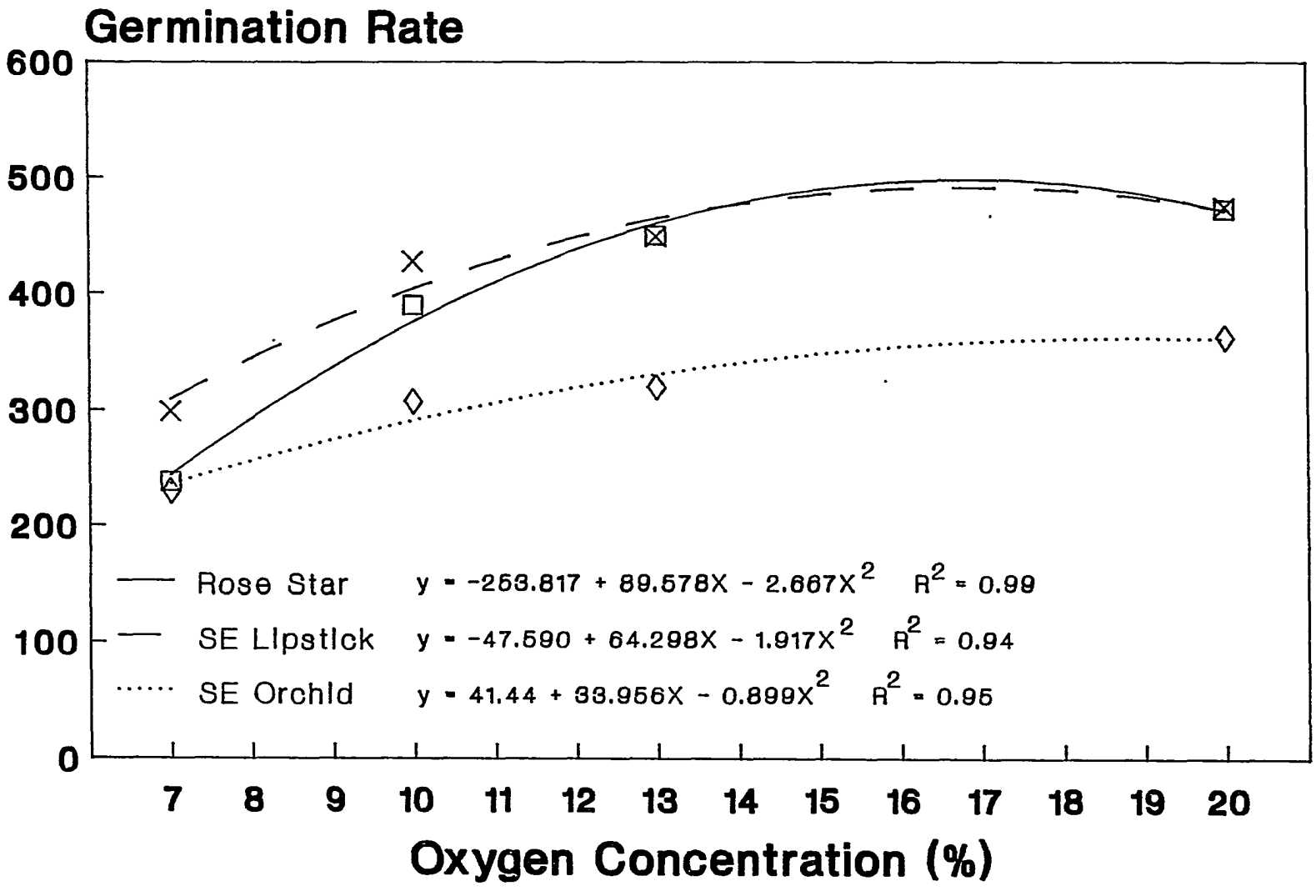
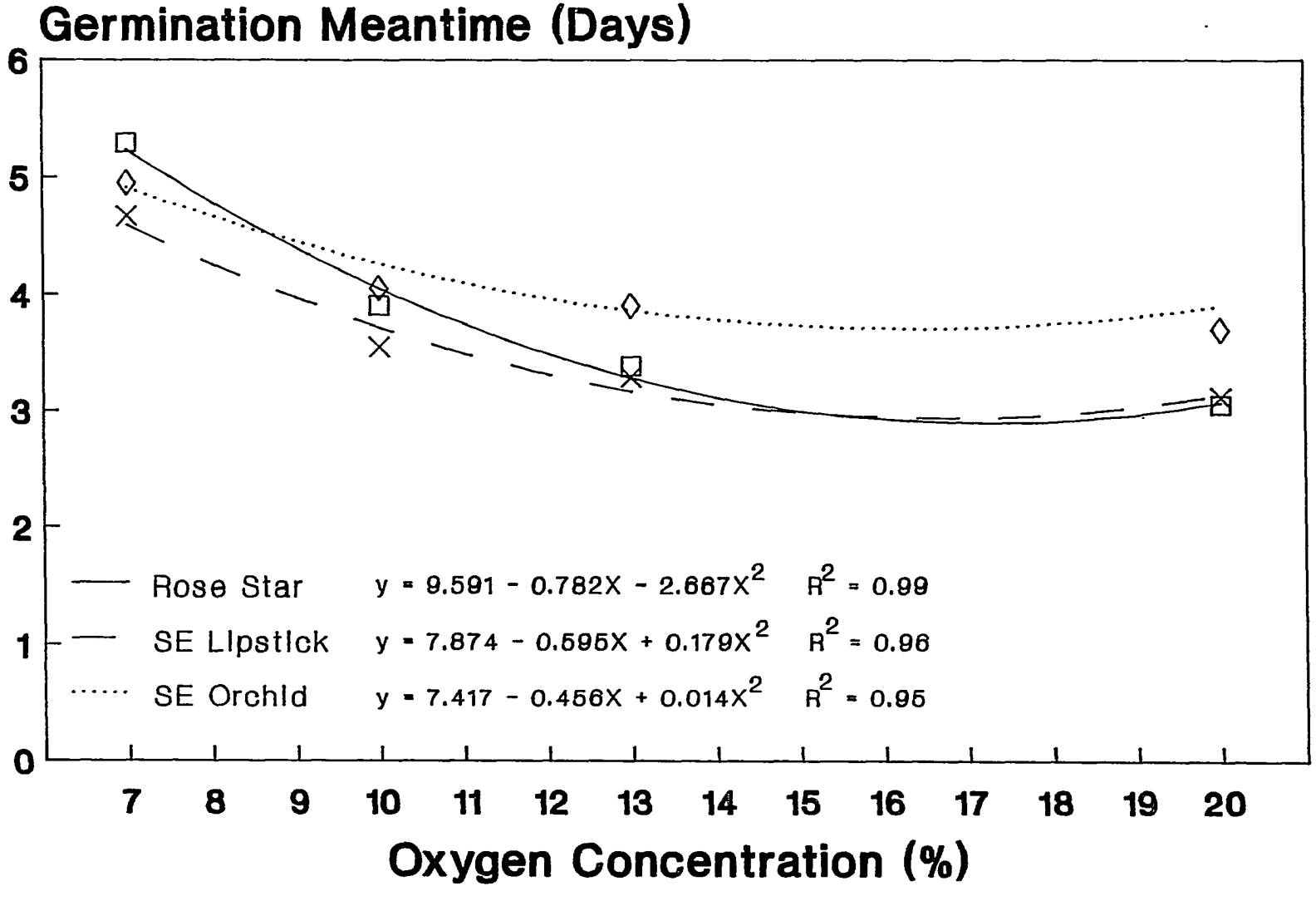

Germination Rate
600
500
400
300
200
100
0
Rose Star y = -253.817 + 89.578X - 2.667X2 R2 = 0.99
SE Lipstick y = -47.590 + 64.298X - 1.917X2 R2 = 0.94
SE Orchid y = 41.44 + 33.956X - 0.899X2 R2 = 0.95
7 8 9 10 11 12 13 14 15 16 17 18 19 20
Oxygen Concentration (%)

Figure 4. Regression lines showing the effect of oxygen concentration on the germination meantime of seeds of the impatiens cultivars 'Rose Star', 'Super Elfin (SE) Lipstick', and 'SE Orchid'. Each symbol represents the mean of 6 observations


Germination Meantime (Days)
6
5
4
3
2
1
0
Rose Star y = 9.591 - 0.782X - 2.667X2 R2 = 0.99
SE Lipstick y = 7.874 - 0.595X + 0.179X2 R2 = 0.96
SE Orchid y = 7.417 - 0.456X + 0.014X2 R2 = 0.95
7 8 9 10 11 12 13 14 15 16 17 18 19 20
Oxygen Concentration (%)

SECTION III. EFFECT OF SEED BURIAL IN PLUG TRAYS ON GERMINATION AND EMERGENCE OF SIX IMPATIENS CULTIVARS

EFFECT OF SEED BURIAL IN PLUG TRAYS ON GERMINATION AND EMERGENCE OF SIX IMPATIENS CULTIVARS

Paul T. Karlovich, Richard J. Gladon, and David S. Koranski

ABSTRACT

Six impatiens cultivars (Impatiens wallerana Hook. f.) were germinated on the surface of or buried 2 mm deep in three soil media in plug trays. Three soil medium-moisture levels were studied. When seeds were placed on the surface of the plug soil medium, germination percentage was not affected by either the soil medium type or the soil medium-moisture level, with the exception of 'Impulse Rose' which had a reduced germination percentage at the greatest soil medium-moisture level. In contrast, burial of impatiens significantly reduced germination and emergence of all cultivars. 'Super Elfin Orchid' and 'Super Elfin Pink' germination percentages were higher than those for the other cultivars, and their ability to germinate under low-oxygen environments may be advantageous in plug-production systems. Emergence from the plug soil medium was reduced more than germination in all cases, and this observation reveals that post-germinative events are curtailed more than are germinative events when impatiens seeds are buried in the soil medium in a plug tray.

INTRODUCTION

During the course of low-oxygen studies on the germination of impatiens seeds, we observed that impatiens seeds would not germinate in low-oxygen environments (<3% O_2) created with nitrogen and oxygen gases (Karlovich et al., 1988). Conversely, most impatiens cultivars would germinate readily in the low-oxygen environment created by submergence in water (see section I). (The maximum oxygen concentration in water ranges from 0.0489 to 0.0209 $ml \cdot ml^{-1}$ H_2O (70 to 30 $mg \cdot l^{-1}$), at 0 to 50C, respectively (Taylor, 1978).) Morinaga (1926) reported that carnation, chamomile, wormwood, timothy, white clover, and celosia seeds germinated under water but not in gaseous atmospheres devoid of O_2. The response of impatiens seeds to low-oxygen environments is of particular interest with respect to plug-production systems. Such systems are characterized by a high soil moisture level and a correspondingly low soil air space. Milks et al. (1989) have estimated that under the best conditions most plug cells have 4% soil air space at container capacity. Therefore, it is likely that a seed buried in a plug cell is subjected to a low-oxygen environment. This experiment examined the germination of seeds of six impatiens cultivars placed on top of a plug soil medium or buried 2 mm deep in the soil medium to determine the response of impatiens seeds to a low-oxygen/high moisture environment.

MATERIALS AND METHODS

Seeds of impatiens cultivars 'Rose Star' (H.G. German Seeds, Smethport, PA), 'Impulse Rose' (Sluis & Groot, Englewood CO), and 'Super Elfin Orchid', 'Super Elfin Pink', 'Super Elfin Orange', and 'Super Elfin Red' (Ball Seed Co., West Chicago, IL) were germinated in 392-cell plug trays (Landmark Plastic Corp., Akron, OH) either on top of the soil medium or buried 2 mm below the surface of the soil medium. The experiment was conducted in three types of soil media: a coarse silica sand, grade no. 16; a fine silica sand, grade no. 430 (UniMin Corp., Le Sueur, MN); and a peat-lite mix (35% Sphagnum peat moss (<0.625 cm), 35% Hypnum peat moss (<0.625 cm), 30% sand-finish perlite (v/v/v)) amended with 3.0 $kg \cdot m^{-3}$ $CaCO_3$, 1.0 $kg \cdot m^{-3}$ superphosphate (0N-19.8P-0K), and 0.6 $kg \cdot m^{-3}$ Esmigram® micronutrient mix. Three soil medium-moisture levels were created, after complete saturation of the medium, by: 1) draining the tray for 30 minutes by using blotter paper to wick water from the medium; 2) allowing the tray to drain to container capacity; or 3) placing the tray in 0.5 cm of water that created a moisture level greater than container capacity. Attempts to quantify the resultant percentage airspace were not successful, and the moisture treatments were designated as drained, container capacity, and greater than (>) container capacity. Seeds were buried by pushing the seed into the saturated medium with a toothpick marked at 2 mm. The plug trays were cut to 7 by 11 plug cells, and they fit into plastic trays (23 × 5 × 5 cm Desk Organizer, Rubbermaid, Inc, Wooster, OH). Each plastic tray was covered with plastic wrap (Saran™) to prevent evaporation. Within each tray,

alternate rows were buried, starting with the outside row. This resulted in 6 rows of buried seeds and 5 rows of seeds that were placed on top of the soil medium.

After seven days, germination counts were taken on the seeds placed on top of the soil medium, and seedling emergence from the soil medium was counted on the buried seeds. Germination was counted if the radicle had protruded through the seed coat (Bewley and Black, 1985), and seedling emergence was counted if any seed part was visible. Buried seeds that did not emerge from the soil medium were excavated and checked for radicle emergence. Percentage germination for buried seeds was calculated by combining the number of emerged seedlings with the number of seeds that had germinated but had not emerged from the soil medium. Occasionally, a seed could not be found. In this case, the data for the seed was dropped from the data set.

This experiment was conducted in a growth chamber at 25C under continuous irradiance from cool-white fluorescent lamps at approximately 25 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux. All of the seeds placed on top of the soil medium had "emerged" when they germinated, and the emergence data only was important with buried seeds. Therefore, the experiment was analyzed in two ways: 1) as a split-split plot design for percentage germination data, with the soil medium as the main plot, and cultivar and moisture level as the split plot, and planting depth nested within moisture level, and 2) as a split plot design for percentage emergence data, with the soil medium as the main plot, and cultivar and

moisture level as the split plot. The experiment was replicated three times over time.

RESULTS

Analysis of percentage germination data for the entire experiment revealed that the depth treatment was the most significant effect (Table 1). All other main effects were significant, and all interactions were highly significant with the exception of the medium by cultivar by soil medium-moisture level interaction. Examination of the mean squares showed that, although many highly significant sources of variation occur, most of the effects can be accounted for by the cultivar and soil medium-moisture level main effects, and by the cultivar by depth and soil medium-moisture level by depth interactions.

Because of the large effect of planting depth, analysis of the experiment was divided by depth, and each depth was analyzed separately (Table 2). This revealed that the effect of depth was due almost entirely to the burial treatment. Seeds germinated on top of the soil medium showed significant cultivar differences and a small, but significant cultivar by soil medium-moisture level interaction. This interaction was significant because of the poor germination of 'Impulse Rose' at the wettest soil medium-moisture level (> container capacity) (Table 3). The other cultivars showed no significant germination percentage differences as the soil moisture level increased. Germination percentages of the cultivars on the surface of the medium were all above 90% except for 'Impulse Rose' (Table 4), and these germination percentages were similar to those on blotter paper (data not shown). Soil medium type and soil medium-moisture level did not affect percentage germination of seeds placed on top of the soil medium.

When the seeds were buried, cultivar differences for percentage germination and percentage emergence were found (Tables 2 and 4). 'Super Elfin Orchid' (SEO) was least affected by burial in the plug soil medium, but even so, SEO germination percentage was low compared with germination on the surface of the soil medium. Although 80% of the buried SEO seeds germinated, only 28% of the seeds had emerged from the soil medium after seven days. With buried seed, the soil medium type had a small effect (significant at the 10% level) on percentage germination, but the soil medium type had a highly significant effect on percentage emergence (Table 2). Germination and emergence percentages were highest in coarse sand and lowest in fine sand (Table 4). Soil medium-moisture level had a larger effect than soil medium type on germination and emergence. Drained soil medium resulted in the highest germination and emergence percentages, and these progressively and sharply declined at soil medium moisture levels of container capacity and > container capacity (Table 4).

Buried seed had high significance for most interactions (Table 2). The soil medium-moisture level by soil medium interaction showed that germination percentage in fine sand is initially lower, but the decline at higher soil medium-moisture levels is not as sharp and to a lesser magnitude than for coarse sand and the peat-lite mix (Figure 1a). The plateau of percentage germination in fine sand between container capacity and > container capacity is interesting, particularly because coarse sand and the peat-lite mix do not show this plateau. The germination plateau occurred because SEO and Super Elfin Pink (SEP) were not affected as greatly by increasing soil medium-moisture in fine sand as in coarse sand

and the peat-lite mix (Figure 2). Surprisingly, SEO germination percentage is greatest in fine sand under the wettest conditions (> container capacity).

The soil medium by soil medium-moisture interaction for percentage emergence showed that emergence from the soil medium was reduced progressively as the soil medium-moisture increased (Figure 1b). The interaction was due primarily to the large differences in emergence percentage under drained conditions. Fine sand had poor emergence regardless of the soil moisture level, but coarse sand had over 60% emergence when it was drained.

The cultivar by soil medium-moisture level interaction was highly significant for percentages of germination and emergence (Table 2). An increase in the soil medium-moisture level reduced germination and emergence of all cultivars (Figures 3a,b). SEO germination and emergence percentage was higher at all moisture levels than the other cultivars, and 'Rose Star' and 'Super Elfin Orange' germination percentage reductions were largest (Figures 3a,b). 'Impulse Rose', which had the poorest germination percentage in general (Table 4), also had the poorest germination and emergence percentages across all moisture levels.

Germination percentage among cultivars was affected differently by the soil medium type (Figure 4a). Most cultivars had the poorest germination in fine sand. But SEO and SEP had the poorest germination in coarse sand and the peat-lite mix, respectively. In contrast to germination percentage, the emergence percentage was greatest in coarse sand and lowest in fine sand for all cultivars (Figure 4b).

DISCUSSION

Simmonds (1980b) reported that burial of impatiens seed 0.5 cm deep improved seedling establishment over that of seeds sown on the surface of the medium. His conditions varied considerably from those used in this study. The most important differences were the height of the container (a 10-cm tall container compared with a 2.5-cm plug cell) and the cultural environment (unprotected in a greenhouse compared with protection by plastic wrap). We feel that our conditions are closer to the conditions that are necessary for successful plug production (Koranski, 1987; Koranski and Laffe, 1988). Also, our results indicate that seed burial in a plug tray, regardless of the type of the soil medium or the soil medium-moisture level, is detrimental to germination and subsequent growth of impatiens. Germination percentage of SEO and SEP was affected less by burial than were the other cultivars (Table 4), and these results demonstrated that these cultivars have an ability to perform better under low-oxygen/high-moisture environments. This better performance may be advantageous for impatiens seedling production in plug systems, especially when the low-oxygen/high-moisture stress is of a shorter duration than the one that was imposed in this study.

Light is recommended for optimum germination of impatiens (Anon., 1975; Koranski, 1988b) and may have affected germination of the buried seeds. Simmonds (1980a) found that the light requirement for impatiens was small and cultivar-specific. Research in our laboratory showed that the light requirement was cultivar-specific, but that some cultivars had a large, positive response to light (data not presented). Light cannot

be excluded totally as a factor in this experiment, but because light-requiring cultivars (e.g., SEP) germinated much better than in dark germination studies and because cultivars with no light requirement (e.g., 'Rose Star') germinated much worse than in dark germination studies, we feel that the response is due primarily to the low-oxygen environment created by the greater amount of moisture in the soil medium.

Seedling emergence from the medium was reduced more than was the germination percentage (Table 4). Emergence from the soil medium is the result of postgerminative events that have higher oxygen requirements (Bewley and Black, 1985). Low emergence percentages, regardless of other factors, indicate that burial affects postgerminative events more than it does the germinative events, and this most likely is due to low-oxygen stress created by the greater moisture in the soil medium. In this regard, the recommendation for drier soil medium-moisture conditions in Stage II of plug production (a postgerminative stage) seems justified (Koranski, 1988a).

The relatively greater germination percentage in fine sand at the highest soil medium-moisture level is puzzling (Figure 1a). This effect was seen because SEO and SEP germinated well in fine sand (Figure 3a), and the germination percentages of these two cultivars was affected less by the increased soil medium-moisture in the fine sand (Figure 2). We believe that the fine sand would have a lower airspace than the other soil media at all soil medium-moisture levels. If this is true, then it is possible that SEO and SEP have a quadratic-type response to oxygen concentration and that low oxygen levels are less detrimental to the

germination process of these cultivars than higher, but still low, oxygen levels. Support for this idea is found in that the two cultivars with the best buried germination percentage, SEO and SEP, had high germination percentages across all medium-moisture levels in fine sand, but 'Rose Star' and 'Super Elfin Orange', which are more sensitive to burial (Table 4), did not demonstrate an ability to germinate better in fine sand (Figure 4a). Further support for this hypothesis is that emergence percentage, which is affected more by burial than germination percentage (Table 4), always is lowest in fine sand and at increased soil medium-moisture levels (Figures 3b, 4b). Because we were unable to determine accurately the airspace in these media, this hypothesis is speculative.

Seeds placed on the surface of the soil medium germinated well regardless of the soil medium type or soil moisture level, and the germination percentages are similar to those found under the ideal conditions of germination on blotter paper. 'Impulse Rose' was an exception (Table 3). This was a low-quality lot, and these results suggest that low-quality seed lots may be affected adversely by high moisture environments, even when the seeds are placed on the surface of the medium. More research must be conducted before more definitive statements can be made about this observation.

The markedly different germination percentages of seeds on the plug medium surface as compared to those buried showed that every precaution should be taken by the plug producer to insure that impatiens seeds are not buried in the plug medium. The higher germination percentages of SEO and SEP under buried conditions is a desirable characteristic and may be

a selection characteristic in future breeding programs. We caution that some plug producers lightly cover impatiens seed with coarse vermiculite or a similar material, and these results do not apply to this practice. Also, we feel that more research on lot-to-lot variability within these cultivars is necessary to determine the stability of the traits responsible for an improved germination percentage in low-oxygen environments.

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Table 1. Mean squares for ANOVA for percentage germination of impatiens seeds (complete analysis)

Source	df	Percentage Germination
Block (B)	2	229 NS
Medium (M)	2	1,421 *
Error A	4	164
Cultivar (C)	5	13,341 ***
Moisture (MT)	2	11,883 ***
C × MT	10	245 **
M × C	10	253 ***
M × MT	4	918 ***
M × C × MT	20	87 NS
B × M × C × MT	102	76 *
Depth (D)	1	146,537 ***
C × D	5	3,522 ***
M × D	2	810 ***
MT × D	2	10,666 ***
C × MT × D	10	321 ***
C × M × D	10	300 ***
M × MT × D	4	906 ***
M × C × MT × D	20	69 ***
Error B	108	53

NS,*,**,***Nonsignificant(ns) or significant F value at the 5% (*), 1% (**), or 0.1% (***) level, respectively.

Table 2. Mean squares for ANOVA for percentage germination of impatiens seeds on the surface of the medium (Not-buried) and percentage germination and percentage emergence of impatiens seeds buried 2 mm (Buried)

Source	df	Percentage Germination (Not-buried)	Percentage Germination (Buried)	Percentage Emergence (Buried)
Block	2	8 NS	572 NS	53 NS
Medium (M)	2	46 NS	2,186 NS[a]	5,395 ***
Error A	4	14	349	169
Cultivar (C)	5	4,211 ***	12,653 ***	1,628 ***
Moisture (MT)	2	20 NS	22,529 ***	16,966 ***
C × MT	10	60 *	506 ***	430 ***
C × M	10	21 NS	531 ***	240 ***
M × MT	4	22 NS	1,801 ***	3,005 ***
M × C × MT	20	19 NS	138 NS	119 *
Error B	102	21	96	53

[a]Significant F value at the 10% level.

NS,*,**,***Nonsignificant(ns) or significant F value at the 5% (*), 1% (**), or 0.1% (***) level, respectively.

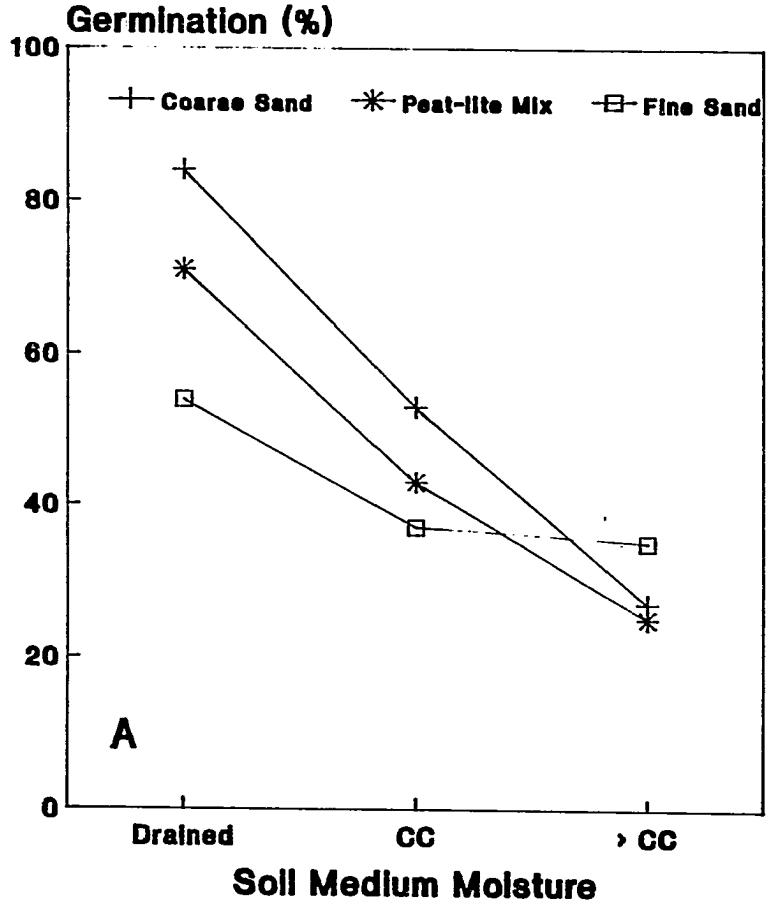
Table 3. Effect of soil medium-moisture level on percentage germination of impatiens seed cultivars placed on top of the plug soil medium

Cultivar	Percentage Germination		
	Medium Moisture Level		
	Drained	Container Capacity	> Container Capacity
Impulse Rose	70	67	59
Super Elfin Orange	93	94	93
Super Elfin Pink	98	98	98
Super Elfin Red	93	94	95
Rose Star	97	97	98
Super Elfin Orchid	93	92	94
$LSD_{0.05} = 3$			

Table 4. Percentage germination of impatiens seeds on the surface of the soil medium (Not-buried) and percentage germination and percentage emergence of impatiens seeds buried 2 mm (Buried) as affected by medium type, cultivar, and soil medium-moisture

Variable	Percent Germination (Not-buried)	Percent Germination (Buried)	Percent Emergence (Buried)
Medium			
Coarse Sand	91	55	25
Peat-lite Mix	90	46	16
Fine Sand	89	42	5
$LSD_{0.05}$	2	4	3
Cultivar			
Super Elfin Pink	98	61	16
Rose Star.	98	41	13
Super Elfin Red	94	49	15
Super Elfin Orange	93	39	15
Super Elfin Orchid	93	80	28
Impulse Rose	65	16	4
$LSD_{0.05}$	2	5	4
Medium Moisture Level			
Drained	91	70	35
Container Capacity	90	44	8
> Container Capacity	90	29	2
$LSD_{0.05}$	2	4	3

Figure 1. Effect of soil medium-moisture level and soil medium type on A) germination percentage and B) emergence percentage of impatiens seeds. Soil medium-moisture levels were created by: 1) draining the tray for 30 minutes by using blotter paper to wick water from the medium (Drained); 2) allowing the tray to drain to container capacity (CC); or 3) placing the tray in 0.5 cm of water that created a moisture level greater than container capacity (>CC)


Germination (%)
100
80
60
40
20
0
Coarse Sand
Peat-lite Mix
Fine Sand
A
Drained
CC
› CC
Soil Medium Moisture

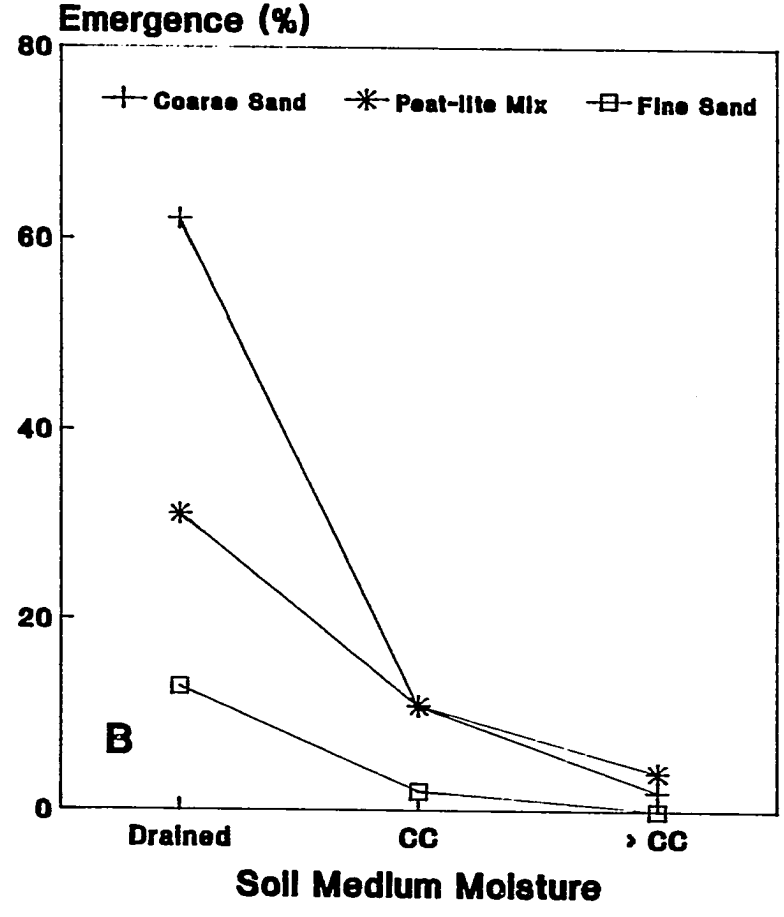

Emergence (%)
80
60
40
20
0
Coarse Sand
Peat-lite Mix
Fine Sand
B
Drained
CC
› CC
Soil Medium Moisture

Figure 2. Effect of soil medium type and soil medium-moisture on germination percentage of impatiens cultivars 'Super Elfin (SE) Pink', 'SE Orchid', 'SE Orange', 'Rose Star, 'SE Red', and 'Impulse Rose'. Soil medium-moisture levels were created by: 1) draining the tray for 30 minutes by using blotter paper to wick water from the medium (DR); 2) allowing the tray to drain to container capacity (CC); or 3) placing the tray in 0.5 cm of water that created a moisture level greater than container capacity (>CC)

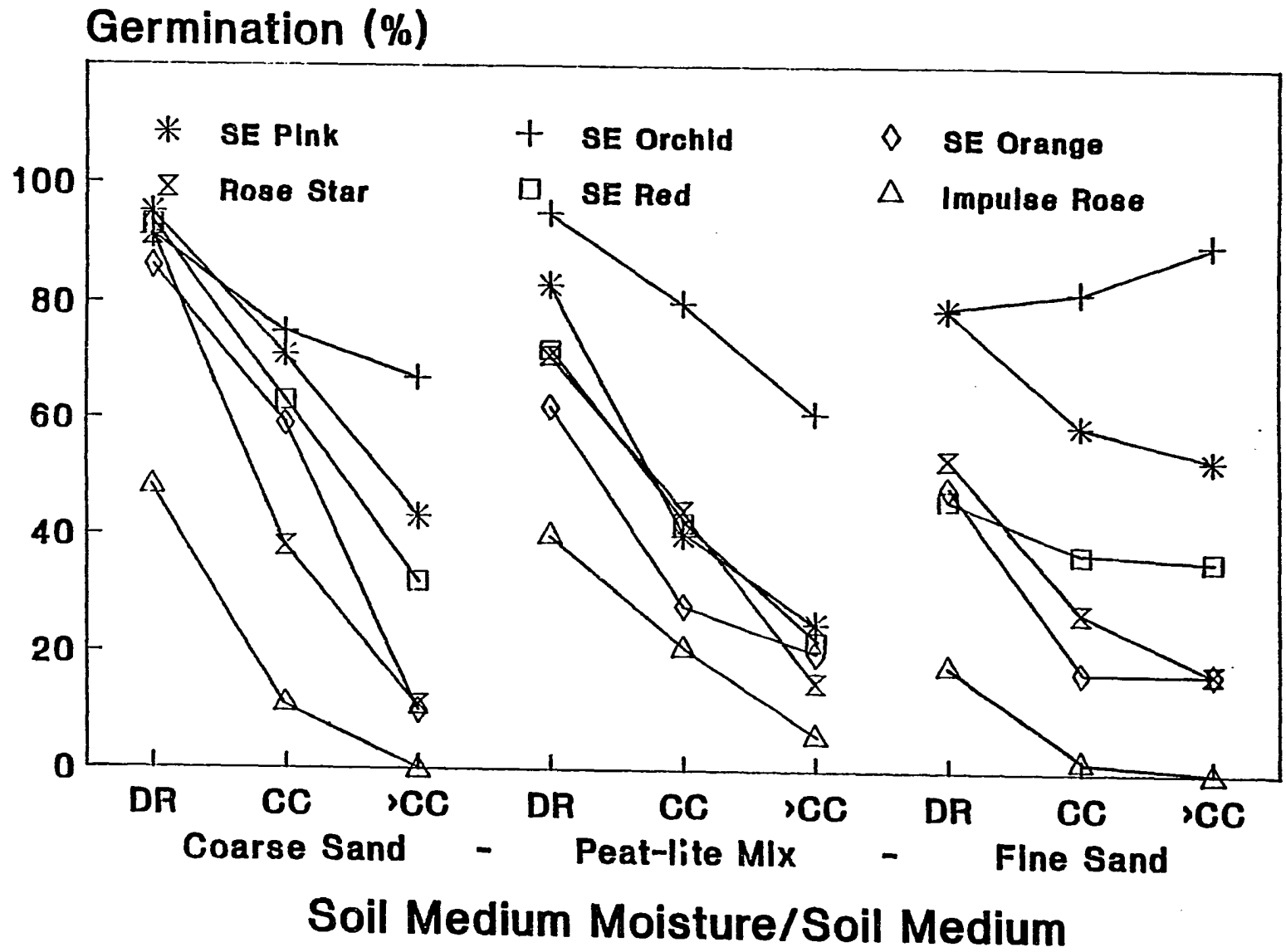
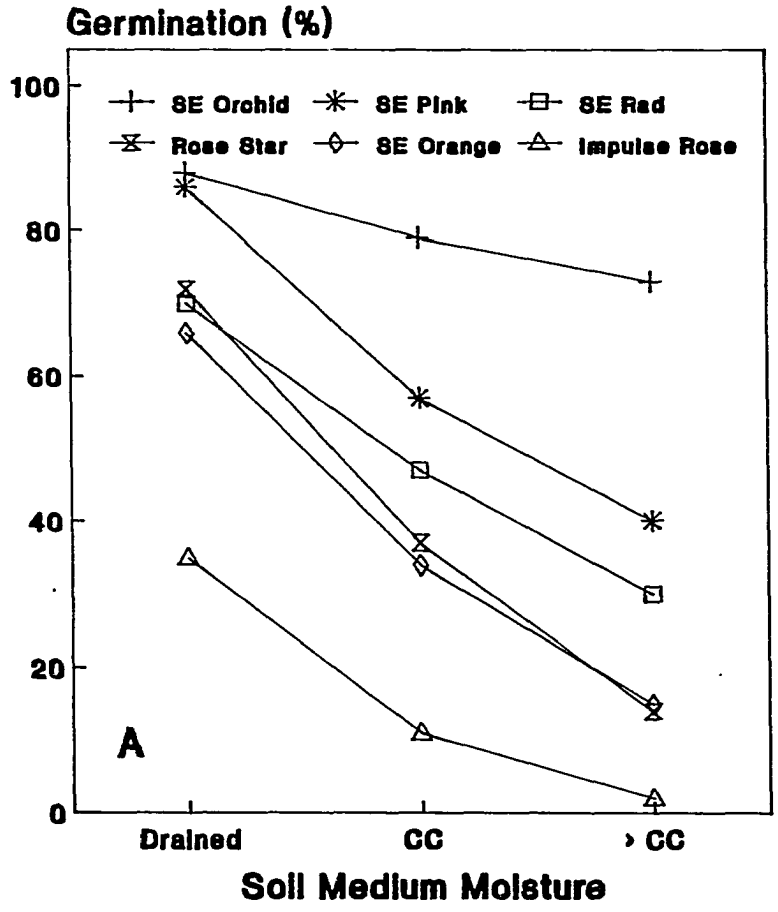

Germination (%)
SE Pink
Rose Star
SE Orchid
SE Red
SE Orange
Impulse Rose
100
80
60
40
20
0
DR
CC
›CC
DR
CC
›CC
DR
CC
›CC
Coarse Sand - Peat-lite Mix - Fine Sand
Soil Medium Moisture/Soil Medium

Figure 3. Effect of soil medium-moisture level on A) germination percentage and B) emergence percentage of the impatiens cultivars 'Super Elfin (SE) Orchid', 'SE Pink', 'SE Red', 'Rose Star', 'SE Orange', and 'Impulse Rose'. Soil medium-moisture levels were created by: 1) draining the tray for 30 minutes by using blotter paper to wick water from the medium (Drained); 2) allowing the tray to drain to container capacity (CC); or 3) placing the tray in 0.5 cm of water that created a moisture level greater than container capacity (>CC)


Germination (%)
SE Orchid
SE Pink
SE Red
Rose Star
SE Orange
Impulse Rose
100
80
60
40
20
0
A
Drained
CC
› CC
Soil Medium Moisture

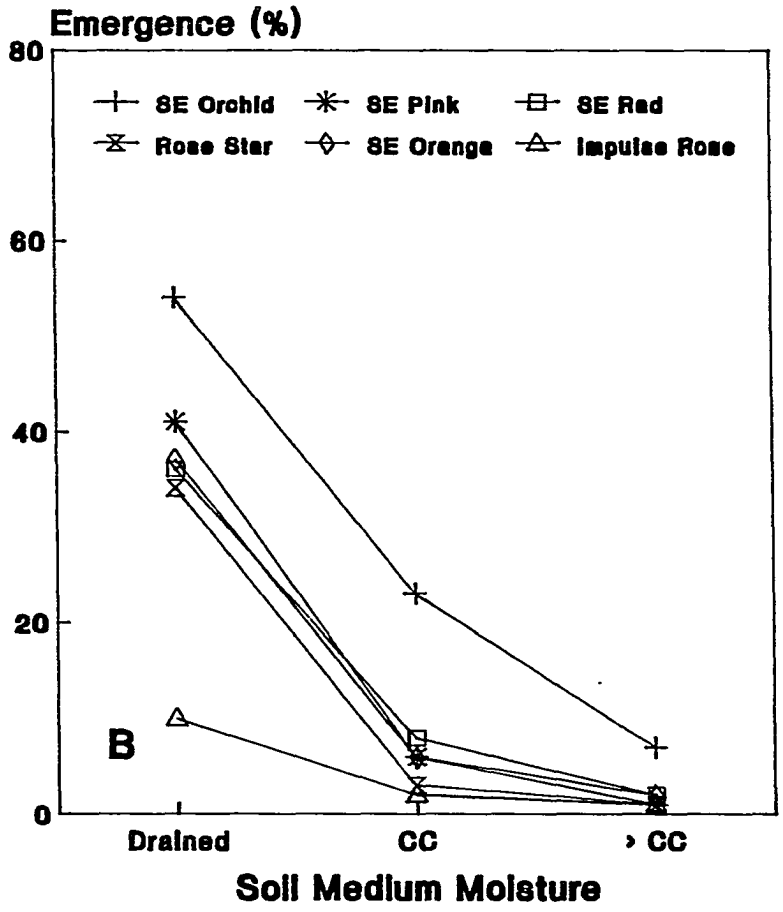
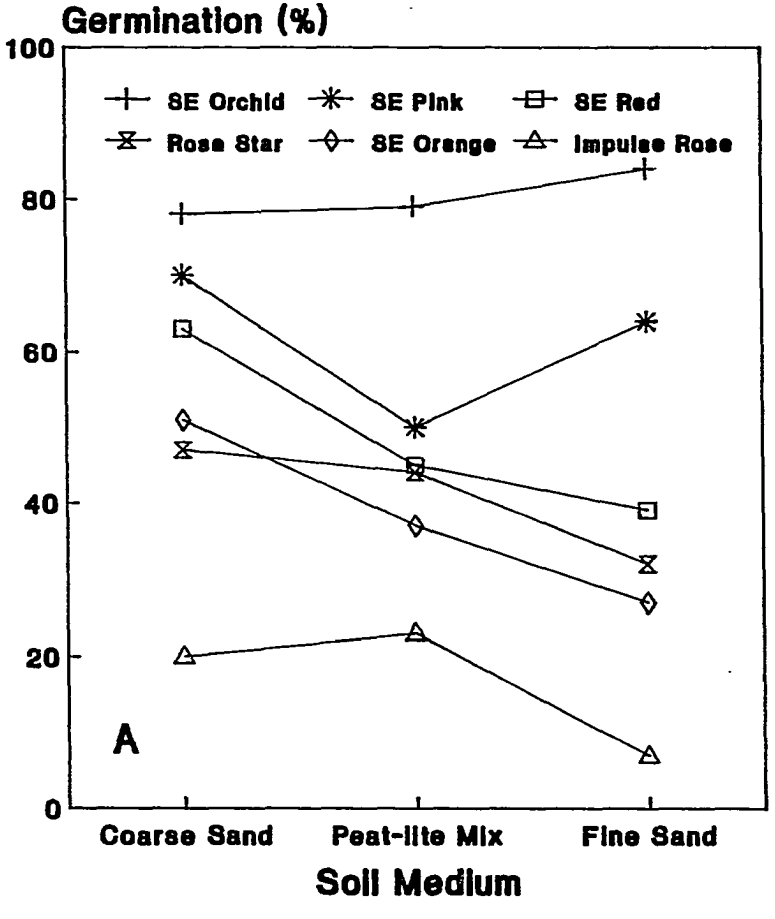
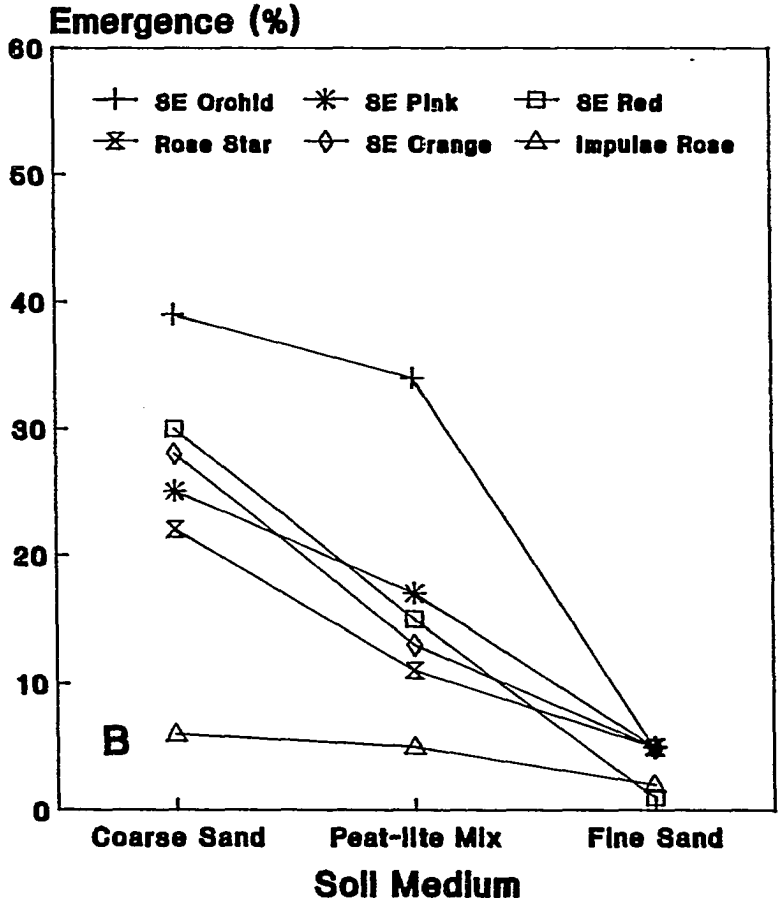

Emergence (%)
SE Orchid
SE Pink
SE Red
Rose Star
SE Orange
Impulse Rose
80
60
40
20
0
B
Drained
CC
› CC
Soil Medium Moisture

Figure 4. Effect of soil medium type on A) germination percentage and B) emergence of the impatiens cultivars 'Super Elfin (SE) Orchid', 'SE Pink', 'SE Red', 'Rose Star', 'SE Orange', and 'Impulse Rose'


Germination (%)
100
80
60
40
20
0
SE Orchid
SE Pink
SE Red
Rose Star
SE Orange
Impulse Rose
A
Coarse Sand
Peat-lite Mix
Fine Sand
Soil Medium


Emergence (%)
60
50
40
30
20
10
0
SE Orchid
SE Pink
SE Red
Rose Star
SE Orange
Impulse Rose
B
Coarse Sand
Peat-lite Mix
Fine Sand
Soil Medium

SECTION IV. ETHANOL, LACTATE, AND MALATE PRODUCTION BY GERMINATING IMPATIENS SEEDS IN NITROGEN AND AIR ATMOSPHERES AND UNDER WATER

ETHANOL, LACTATE, AND MALATE PRODUCTION BY GERMINATING IMPATIENS SEEDS IN NITROGEN AND AIR ATMOSPHERES AND UNDER WATER

Paul T. Karlovich, Richard J. Gladon, and David S. Koranski

ABSTRACT

Seeds of Impatiens wallerana cv. Super Elfin Lipstick were germinated in atmospheres of 100% nitrogen gas or air. Ethanol, lactate, and malate were measured at 0, 4, 8, 12, and 24 hours and at 24-hour intervals for an additional 96 hours. At 120 hours, the nitrogen gas-treated seeds were transferred to air, and the sample removal continued at 24-hour intervals to hour 240. In air, seed ethanol and lactate did not accumulate, but malate increased slowly until hour 72 and then increased rapidly thereafter. Under 100% nitrogen gas, lactate increased to a concentration twice that of air-treated seeds, and ethanol accumulated to a concentration 20 times greater than that of seeds germinated in air. After transfer to air, ethanol and lactate concentrations decreased to levels similar to those of seeds germinated in air. Under nitrogen gas, the concentration of malate in the seeds rose slightly during the first 8 hours, and then it gradually decreased to a concentration that was less than one-half that of seeds germinated in air. Upon return to air, seed malate accumulated steadily, with larger increases concurrent with the onset of germination. In a second experiment, 25 seeds were submerged in either 40 ml or 200 μl of water. No ethanol was detected in the water surrounding the seeds in 40 ml of water, but ethanol accumulated rapidly in the water surrounding the seeds in 200 μl of water. The ethanol concentration reached a maximum at 72 hours, and it then declined steadily.

INTRODUCTION

In earlier studies, we reported that impatiens seeds germinated when submerged in water and that impatiens seeds did not germinate when placed in 0% oxygen (100% nitrogen gas) or in 3% oxygen in nitrogen (see sections I and II). These responses, while seemingly contradictory, have been observed previously (Morinaga, 1926). In research published previously, no attempt was made to explain these differences in response to different methods of imposing oxygen stress.

The general response to reduced oxygen levels is an increase in non-oxidative fermentation (Leblova, 1978; Raymond et al., 1985), and several different end products accumulate. Crawford and Tyler (1969) showed that the roots of flooding-tolerant plants accumulated malate, and flooding-intolerant plants did not accumulate malate. Morohashi and Shimokoriyama (1972a) concluded that malate was being synthesized actively early in the germination process of Phaseolus mungo. Lactate was found to be high initially in seeds of lettuce and rice, two seeds that are tolerant to soaking, and the soaking-intolerant seeds, pea and maize, produced ethanol as the major end product of fermentation (Crawford, 1977). Lactate, succinate, and ethanol accumulated in buckwheat seedlings under anaerobic conditions (Effer and Ranson, 1967).

Two lines of experimental evidence have emerged for the mechanism by which plants tolerate low-oxygen environments. McManmon and Crawford (1971) have theorized that tolerance of low-oxygen conditions is best among plants that limit the pasteur effect. Tolerant plants produce less ethanol and instead produce malate and other organic acids. Pesis and Ng

(1984) reported that low-vigor (accelerated aged) Cucumis melo seeds exhibited an apparent Pasteur effect upon exposure to 100% nitrogen gas, but high-vigor seeds showed no such effect. In contrast, Bertani et al. (1980) concluded that rice is tolerant of anaerobic conditions because it couples a strong alcoholic fermentation to an ability to excrete ethanol to the surrounding medium. Taylor (1942) also attributed the ability of rice to germinate in 0% oxygen to alcoholic fermentation. Smith and Ap Rees (1979) reported that marsh plants did not accumulate malate and depended on alcoholic fermentation during anoxia. Other studies have not shown that malate or lactate accumulate (Rumpho and Kennedy, 1981). Most studies with seeds have shown that they produce considerably more ethanol than lactate or malate and that the ethanol is excreted to the imbibition medium and/or the atmosphere (Bertani et al., 1980; Crawford, 1977; Raymond et al., 1985; Rumpho and Kennedy, 1981). Oryza sativa (Bertani et al., 1980) and Echinochloa crus-galli (Rumpho and Kennedy, 1981) excreted to the imbibition medium 98% and 85%, respectively, of the ethanol that they produced.

Based upon the results of our earlier work, we suspect that, for some as yet unexplained reason, ethanol may be excreted from the seed fast enough to allow the seed to germinate when under water but that for seeds in nitrogen gas, ethanol is not excreted fast enough and germination cannot occur. The objective of this research was to determine if ethanol was accumulating in impatiens seeds placed in 100% nitrogen gas, and to determine if ethanol is excreted from seeds submerged in water.

MATERIALS AND METHODS

'Super Elfin Lipstick' (SEL) (Ball Seed Co., West Chicago, IL) impatiens seeds were exposed to 0% and to 20% oxygen atmospheres by using 99.995% nitrogen compressed gas and compressed air, respectively. Four hundred milligrams of seeds (approximately 650 to 700) were placed on dry, Steel Blue Anchor Seed Germination Blotter paper (Anchor Paper, St. Paul, MN) in 946-ml mason jars. The jars in the 0% oxygen treatment were sealed and then flushed with nitrogen gas, and when the correct atmosphere was reached, the blotter paper was saturated by adding deionized water (>18 megOhm$\cdot$cm^{-1} resistance) from a syringe inserted through a septum in the jar lid. A continuous flow of gas of approximately 275 ml$\cdot$hr^{-1} was maintained throughout the experiment. The jars within each treatment were placed in series, and at each sampling time, the end jar was removed and the seeds were prepared for analysis. Samples were taken at 0, 4, 8, 12, and 24 hr the first day and at 24-hr intervals thereafter. The air treatment was terminated at 120 hr. After 120 hr, the remaining jars in the 0% oxygen treatment were switched to air, and sampling was continued at 24-hr intervals for an additional 120 hr.

In a second, concurrent experiment, SEL seeds were submerged either in a large or a small volume (40 ml or 200 μl, respectively) of deionized water (>18 megOhm$\cdot$cm^{-1} resistance). The 40-ml treatment was 25 seeds in a 125-ml Erlenmeyer flask. The 200-μl treatment had 25 seeds in each of three 1.5-ml microcentrifuge tubes for each sampling time. Sample times have been described above. This experiment was terminated after 168 hr.

Both experiments were conducted in the laboratory at 21C with 4 to 7 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux (inside the jars) from cool-white fluorescent lamps for 8 to 10 hours daily.

At each sample time, seeds from the 0%- and 20%-oxygen treatments were weighed and frozen in liquid nitrogen within two minutes. The frozen seeds were ground immediately in 10 volumes (4 ml) of 0.6N perchloric acid. The sample was centrifuged at 20,000 × *g* for 20 minutes. The supernatant was neutralized with potassium carbonate (5M), and after centrifugation to remove the precipitate, the supernatant was decolorized with 2% (w/v) polyvinylpolypyrrolidone (PVPP). After a final centrifugation to remove the PVPP, the supernatant was analyzed for ethanol, lactate, and malate.

In second experiment, 0.5 ml from the 40-ml treatment and three 200-μl tubes were frozen in liquid nitrogen. This experiment differed from the first experiment in that the imbibition water was sampled for ethanol rather than the seeds.

Ethanol, lactate, and malate were determined by measuring the reduction of nicotinamideadenine dinucleotide (NAD) at 340 nm. The specific conditions were:

Ethanol (Beutler, 1983). Potassium diphosphate buffer (pH 9.0; 114 mM), 1.86 mM NAD, 150 units alcohol dehydrogenase (EC 1.1.1.1), and 1.6 units aldehyde dehydrogenase (EC 1.2.1.5) in a final volume of 2.64 ml.

Lactate (Noll, 1983). Glutamate buffer (pH 8.9; 116 mM), 0.93 mM NAD, 0.9 units alanine aminotransferase (EC 2.6.1.2), and 208

units lactate dehydrogenase (EC 1.1.1.27) in a final volume of 3.0 ml.

Malate (Möllering, 1983). 3 amino-1-propanol buffer (pH 10; 76 mM), 2 mM NAD, L-glutamate (pH 10; 50mM), 94 units malate dehydrogenase (EC 1.1.1.37), and 10 units aspartate aminotransferase (EC 2.6.1.1) in a final volume of 2.0 ml.

RESULTS

The fresh weight of SEL seeds in nitrogen and air atmospheres rose rapidly during the first 8 hours and rose then slowly thereafter until hour 48 (Figure 1). At 72 hours, germination was evident in the air-treated seeds, but this was not yet reflected in the fresh weight. As germination progressed, the fresh weight increased rapidly. This shape of the curve for the air-treated seeds is a classical water-uptake pattern characteristic of many species (Bewley and Black, 1985). The nitrogen-treated seeds showed no signs of germination, and the fresh weight of the seeds remained unchanged. After the nitrogen-treatment was switched to air (at 120 hours), it took an additional 72 hours until the first signs of germination were seen (at hour 192). As with the air-treated seeds, the fresh weight at hour 192 does not yet indicate that germination is occurring. After 192 hours, fresh weight began to increase at a faster rate. The fresh-weight gain at this point was not as fast as the fresh-weight gain for the air-treated seeds at similar times after the first evidence of germination (hour 96 versus hour 216 and hour 120 versus hour 240).

Malate concentration of the SEL seeds in air increased slowly during the first 24 hours and then stabilized during the subsequent 24 hrs (Figure 2). Malate increased slightly in the air-treated seeds when germination began (hour 72) and it rose rapidly thereafter. Initially the malate concentration increased in the seeds in nitrogen gas. The malate concentration peaked at hour 8 and then dropped to a concentration approximately one-half of that found in air at hours 24 and 48. The

malate level continued to decrease slowly until the point at which the seeds were switched to air (120 hours). Upon the change to air, the malate concentration showed a linear increase until hour 192. At this time, the malate concentration began to increase more rapidly, and it indicated increased seed germination. As it was seen in the fresh weight changes, the malate concentration at similar times from the start of germination was not as high as it was in the seeds held in air continuously.

Lactate production by seeds germinated in air and in nitrogen gas was difficult to monitor, but in general, the lactate in the seeds in nitrogen gas rose after 8 hours to a concentration 2 to 4 times higher than the concentration of the seeds held in air (Figure 3). Lactate remained at an increased concentration until the seeds were switched to air. Within 24 hours after the switch to air, lactate decreased to a concentration similar to that of the seeds germinated in air.

Ethanol concentration in the seeds that germinated in air did not change significantly over the course of this experiment (Figure 4). In contrast, the ethanol concentration in the seeds that germinated in 100% nitrogen increased steadily until it stabilized between hours 96 and 120. Within 24 hours after the switch of the seeds from nitrogen to air, ethanol decreased to a concentration similar to that of the seeds germinated in air.

In the second experiment, the water that surrounded the seeds submerged in 200 μl of water rapidly accumulated ethanol to an elevated concentration (Figure 5). The ethanol concentration peaked at 72 hours

and steadily declined thereafter. No ethanol was detected in the water that surrounded the seeds germinated in 40 ml of water.

DISCUSSION

In this experiment, lactate accumulated during the early hours of anaerobiosis and then stabilized. Ethanol accumulated steadily from the beginning of the experiment and did not stabilize until hour 96. Malate declined in seeds during anaerobiosis. These results are consistent with the conclusion of Bertani et al. (1980). Davies et al. (1974) reported that the production of lactate under anaerobic conditions was minimized by a decrease in the pH and by an elevated concentration of adenosine triphosphate and pyruvate that inhibit lactate dehydrogenase and stimulate pyruvate decarboxylase. This would cause a switch from lactate to ethanol production. Our study found that the two metabolites initially accumulated simultaneously under anaerobiosis, but lactate production either was stopped or reached a steady-state level after the early hours of imbibition. In other studies lactate was not excreted to the imbibition medium (Bertani et al., 1980), and we assume that it was not excreted from the impatiens seeds used in this study.

The decrease in seed ethanol and lactate concentrations after anoxic conditons were removed has been observed in plants as they switch from anaerobic to normal aerobic respiration (Cameron and Cossins, 1967; Cossins, 1978; Woodstock and Taylorson, 1981). The increase in seed malate concentration after the transfer to air also has been associated with an increased activation of the tricarboxylic acid cycle (Morohashi and Shimokoriyama, 1972b).

We have no explanation for the erratic pattern of lactate accumulation in the seeds that were germinated in 100% nitrogen. Ethanol

and malate were measured in the same extract, and there was no problem with their measurement. We can attach no significance to the 24-hour periodicity of the lactate concentration in this treatment.

Our results support the hypothesis that ethanol accumulation in the seed may be responsible for the failure of seeds to germinate when they were exposed to 100% nitrogen gas. The rapid and large excretion of ethanol to the imbibition medium of the seeds submerged in 200 μl of water showed that impatiens rapidly excrete ethanol to the imbibition medium. The seeds submerged in 200 μl of water did not germinate under these conditions, and we attributed this to the concentration of ethanol in the imbibition water, which would reduce the rate at which ethanol was excreted from the seed. The seeds in 40 ml of water germinated, and no ethanol was detected in the imbibition water. The fact that no ethanol was found in this treatment does not imply that ethanol was not being produced. The 40 ml treatment represents a 200-fold water volume increase of the medium surrounding the seeds, and had the same amount of ethanol been present in this treatment as was found in the 200-μl water treatment, it would have been too dilute to be detected by this assay. If it is assumed that the seeds in nitrogen gas are able to excrete ethanol to at least a similar extent as the seeds under water, then the seeds may have produced more ethanol than that which was found in the seeds. Even a small increase in the resistance to ethanol excretion may cause ethanol to accumulate to inhibitory levels.

The theory proposed by McManmon and Crawford (1971) is an explanation of flooding tolerance of certain plant species. In our

studies, all of the impatiens cultivars tested were tolerant of submergence in water and exposure to gaseous, low-oxygen environments. The conclusion of Bertani et al. (1980) addressed the fact that some seeds have developed a greater tolerance of anoxia, and they now possess the ability to germinate under anaerobic conditions. Submerged seeds of various impatiens cultivars have demonstrated this ability to varying degrees (Section I).

Whether or not ethanol accumulates to high enough concentrations to prevent impatiens seed germination in nitrogen gas but not when submerged in water is still an open question, but our results do not contradict this hypothesis. Additional research must be conducted before conclusive evidence for such an occurrence can be verified.

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Figure 1. Fresh-weight gain of 400 mg (650 to 700 seeds) of 'Super Elfin Lipstick' impatiens seeds exposed to 100% nitrogen gas or to air. After hour 120, the 100% nitrogen treatment was switched to air (indicated by the arrow). Vertical bars represent one standard error of the mean, and points with no bar had standard errors that were too small to appear outside of the symbol

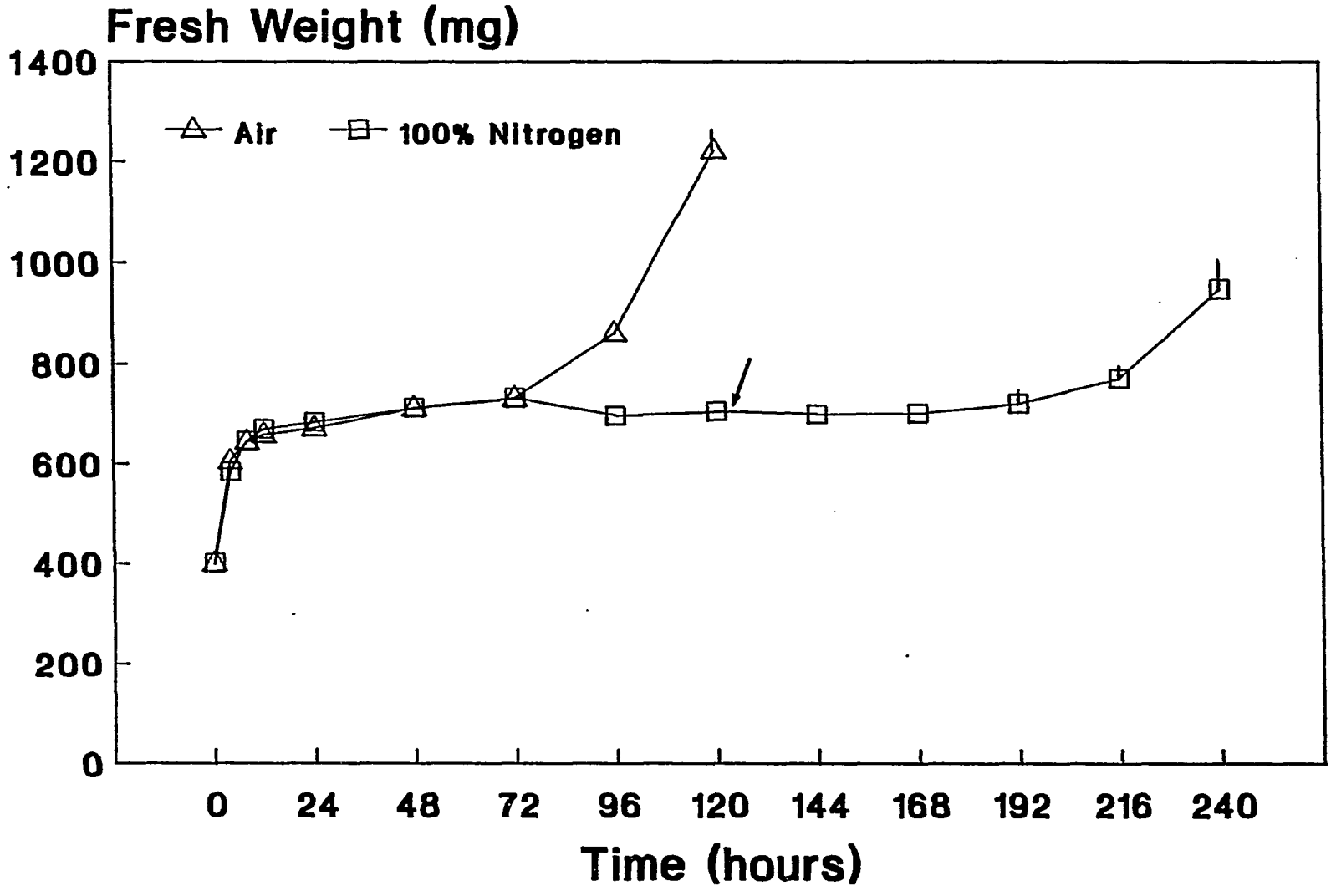

Fresh Weight (mg)
1400
1200
1000
800
600
400
200
0
Air
100% Nitrogen
0
24
48
72
96
120
144
168
192
216
240
Time (hours)

Figure 2. Malate concentration in 'Super Elfin Lipstick' impatiens seeds exposed to 100% nitrogen gas or to air. After hour 120, the 100% nitrogen treatment was switched to air (indicated by the arrow). Vertical bars represent one standard error of the mean, and points with no bar had standard errors that were too small to appear outside of the symbol

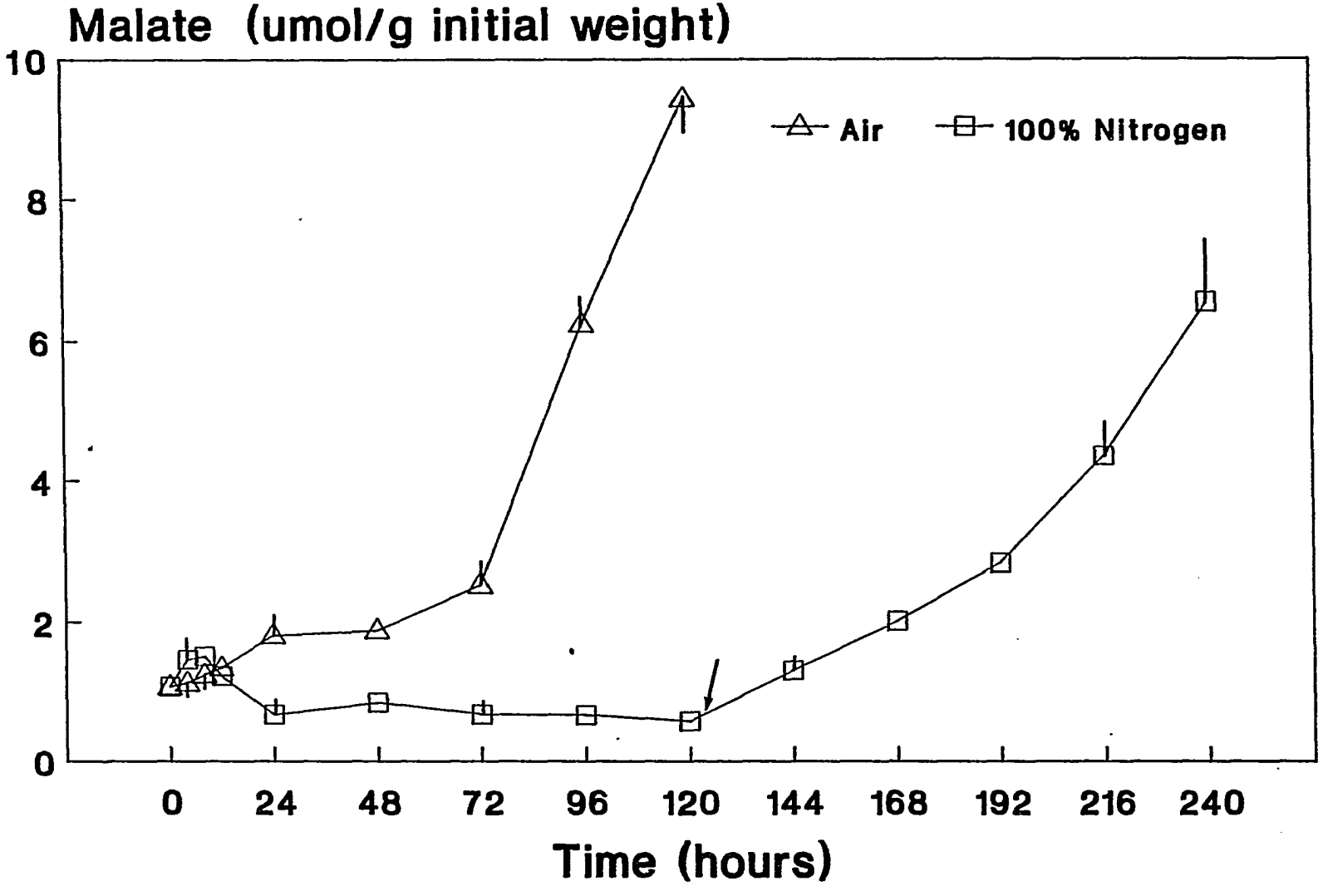

Malate (umol/g initial weight)
10
8
6
4
2
0
Air
100% Nitrogen
0
24
48
72
96
120
144
168
192
216
240
Time (hours)

Figure 3. Lactate concentration in 'Super Elfin Lipstick' impatiens seeds exposed to 100% nitrogen gas or to air. After hour 120, the 100% nitrogen treatment was switched to air (indicated by the arrow). Vertical bars represent one standard error of the mean

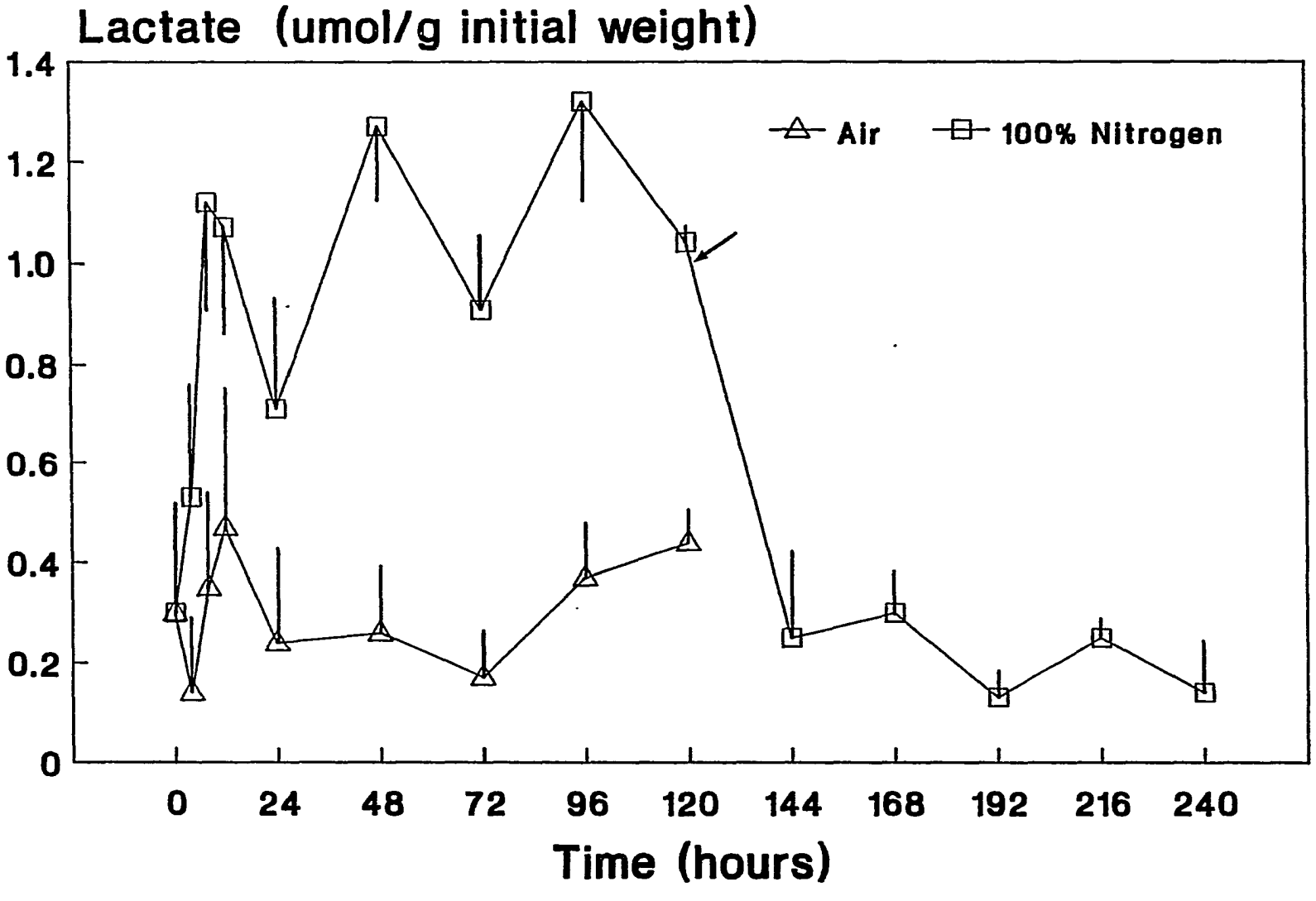

Lactate (umol/g initial weight)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Air
100% Nitrogen
0
24
48
72
96
120
144
168
192
216
240
Time (hours)

Figure 4. Ethanol concentration in 'Super Elfin Lipstick' impatiens seeds exposed to 100% nitrogen gas or to air. After hour 120 the 100% nitrogen treatment was switched to air (indicated by the arrow). Vertical bars represent one standard error of the mean, and points with no bar had standard errors that were too small to appear outside of the symbol

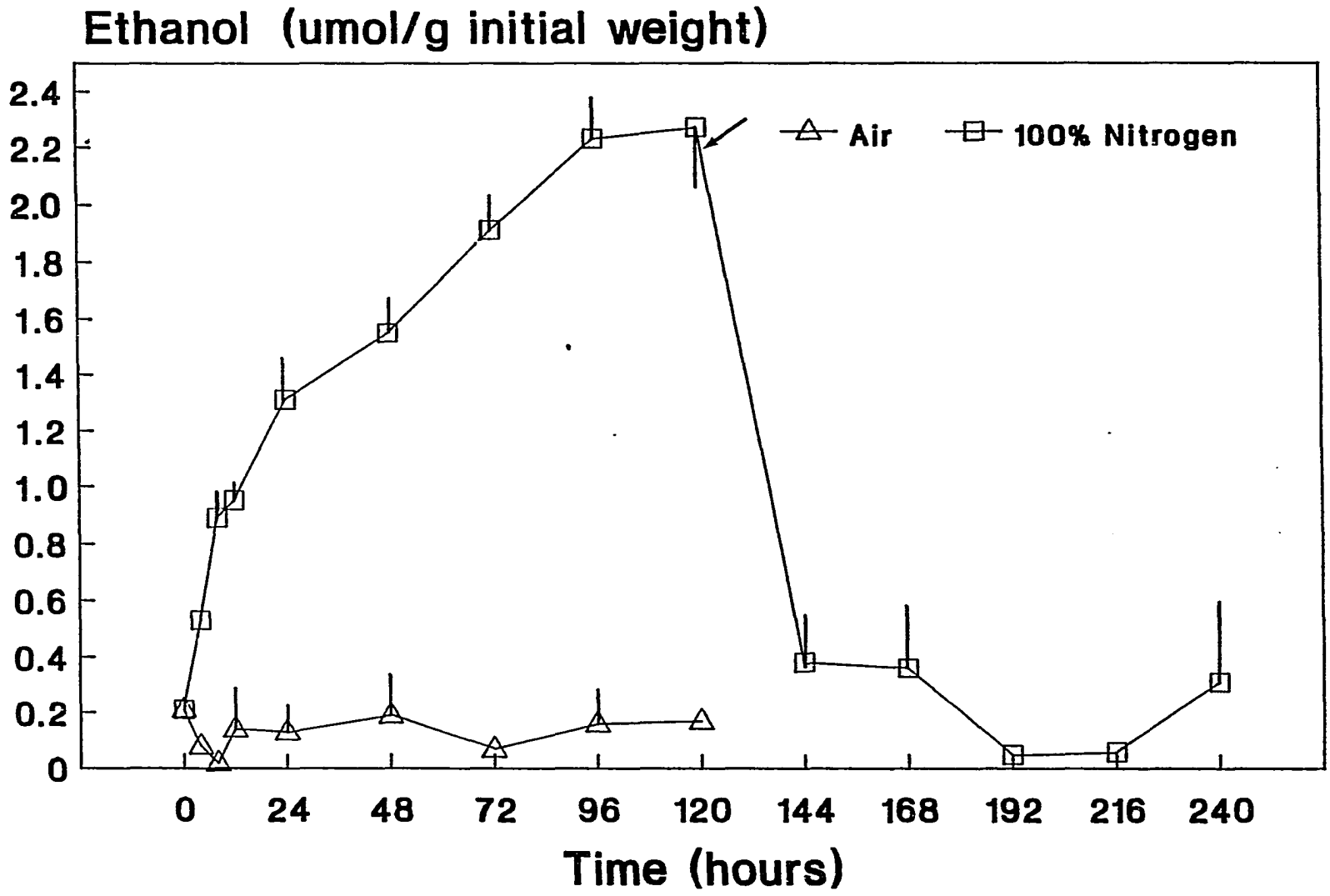
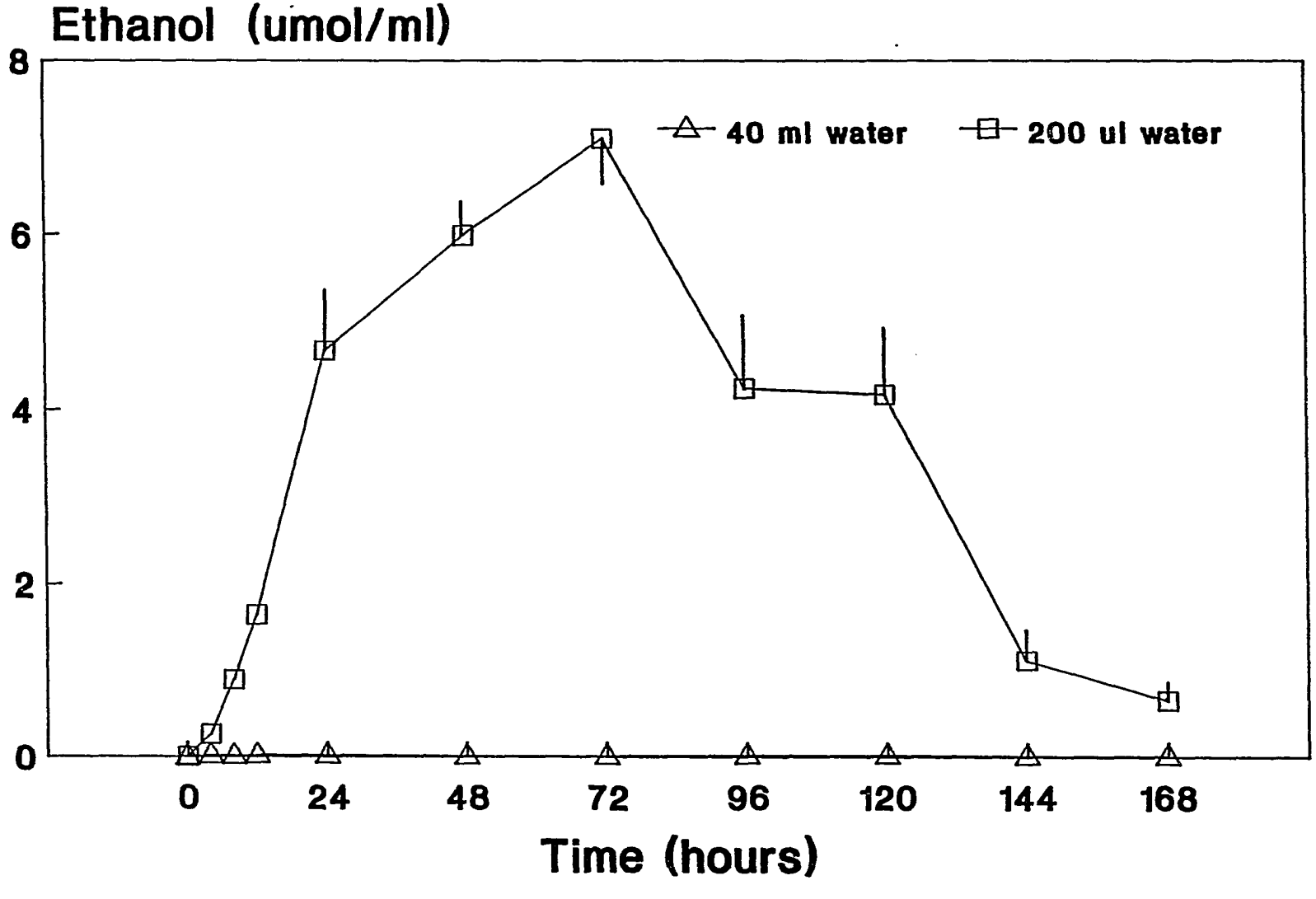

Ethanol (umol/g initial weight)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Air
100% Nitrogen
0
24
48
72
96
120
144
168
192
216
240
Time (hours)

Figure 5. Ethanol concentration in the imbibition water surrounding 25 'Super Elfin Lipstick' impatiens seeds in 200 μl or 40 ml of water. Vertical bars represent one standard error of the mean, and points with no bar had standard errors that were too small to appear outside of the symbol


Ethanol (umol/ml)
8
6
4
2
0
40 ml water
200 ul water
0
24
48
72
96
120
144
168
Time (hours)

SUMMARY AND DISCUSSION

The response of impatiens seeds to oxygen depended upon the system that was used to impose the stress. When low-oxygen stress was imposed by submerging seeds in water, all of the cultivars that were tested could germinate, at least to some degree, in seven days. Several of the cultivars germinated nearly as well under water as did their blotter-paper controls. The response of these cultivars was of interest because of the possibility that these cultivars would perform better under an oxygen stress caused by the increased moisture conditions encountered in plug production systems. In addition, these results showed that, although the cultivars were similar under ideal conditions (on blotter paper), under severe conditions (submerged) the impatiens cultivars had quite variable germination percentages, germination rates, and germination meantimes.

Exposure of impatiens seeds to progressively lower oxygen atmospheres revealed that impatiens germinate best at an oxygen concentration of 20%. Exposure of impatiens seeds to 7% oxygen for even one day before transfer to 20% oxygen delayed germination. No seeds germinated in seven days in oxygen concentrations of 3% or less. The reason for impatiens seed germination under water but not in the low-oxygen gaseous treatments was puzzling and did not provide an answer to the question of what level of oxygen the seed might be exposed to in a plug tray.

It was interesting to note that 'Super Elfin Orchid' seeds, which germinated under water as fast and to the same percentage as did seeds on

blotter paper, germinated poorly in the flow-through system used to test the effects of the different oxygen concentrations. The only explanation that seems feasible for this response is that seeds of 'Super Elfin Orchid' were sensitive to the 275 $ml \cdot hr^{-1}$ flow rate through the system. Possibly the seed factors that allow 'Super Elfin Orchid' to germinate so well under water contribute to the poor response of this seed to the flowing-gas system. Indeed, several tests with a static 20% oxygen concentration caused no inhibition of germination.

In order to determine whether or not seeds in plug trays would respond like the seeds in the oxygen atmosphere experiment or like the seeds in the submergence study, a seed-burial experiment was designed. The results of this study showed that regardless of the type of soil medium and the soil medium moisture level, germination was reduced when seeds were buried (2 mm depth) as compared with seeds placed on the surface of the soil medium. Buried seeds had little chance of emerging from the medium. Based on these results, it is likely that buried seeds never would make usable plugs because of the delays, both in germination and in emergence caused by burial. The inability of buried seeds to germinate and to emerge from the soil medium seems to be an oxygen-associated effect, although there may be an effect of light as well. The germination of seeds placed on the surface of the soil medium was not affected by soil medium type or by soil medium moisture level. The one exception to this finding was a low-quality seed lot, and this seed lot germinated poorly at the highest soil medium moisture level. At least with high-quality seed lots, it seems that, as long as the seeds remain

on the surface of the plug soil medium, they are not subjected to severe oxygen stress.

Additional investigations into the ability of impatiens seeds to germinate under water, but not in 100% nitrogen gas, were conducted. To explain the contradictory results of the germination studies in low-oxygen atmospheres and under water, I hypothesize that seeds under water can excrete ethanol fast enough to prevent ethanol toxicity from inhibiting germination, while seeds exposed to 100% nitrogen gas accumulate ethanol to inhibitory levels. Ethanol accumulated in impatiens seeds in 100% nitrogen but did not accumulate it in seeds germinating in air. The ethanol concentration in seeds that germinated in 100% nitrogen increased steadily before it stabilized at 96 hours. Lactate also accumulated under anaerobic conditions, mostly in the early hours of germination, to levels 2 to 4 times higher than those found in seeds that germinated in air. Malate, after a small, early increase, declined during anaerobic conditions.

Impatiens seeds that germinated in a relatively large volume of water (40 ml) did not germinate when a similar amount of seeds were placed in a small volume of water (200 μl). The ethanol concentration in the imbibition water was measured in both cases. In a small volume of imbibition water, large amounts of ethanol accumulated, but no ethanol was found in the larger volume of imbibition water. Taken together, ethanol accumulation in seeds in 100% nitrogen gas and in a small water volume lends support to the hypothesis that ethanol may be excreted faster under water than in gaseous low-oxygen environments. Additional

research needed to support this hypothesis is needed.

The research that was conducted for this dissertation indicated that impatiens seeds are sensitive to the oxygen concentration to which they are exposed during the germination process. The cultivar-specific responses that were observed in these studies showed that more research into cultivar differences would be beneficial. In addition, more lot-to-lot comparisons need to be done in order to investigate whether or not cultivar differences are caused by seed production and handling differences or by genetic differences. Further work also needs to be done on the effects of seed aging and the seed coat as factors that cause a response to low-oxygen environments.

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APPENDIX A: GERMINATION OF IMPATIENS SEEDS UNDER LIGHT AND DARK CONDITIONS

Procedure

Seeds of twelve cultivars of impatiens were germinated under laboratory lighting (4 to 7 $\mu mol \cdot sec^{-1} \cdot m^{-2}$ photosynthetic photon flux from fluorescent lamps) for 10 hours daily or in darkness. Forty seeds per treatment were placed on water-saturated blue blotter paper in 100 × 15 mm plastic petri dishes. The petri dishes were wrapped with parafilm and dark germinated seeds also were wrapped with aluminum foil. All steps in the initial handling of the seeds for the dark treatment were done in a darkroom in absolute darkness. The experiment was conducted in the laboratory at 21C. Germination counts (radicle emergence) were taken after seven days.

RESULTS

Cultivar	Reps	Germination (%) Light	Reps	Germination (%) Dark
Rose Star	3	95	3	96
Super Elfin Lipstick	5	98	5	92
Accent Pink	3	97	3	80
Accent Salmon	3	89	2	61
Super Elfin Orchid	5	85	5	71
Super Elfin Orange	3	90	3	66
Accent Rose (old)	3	94	3	61
Accent Rose (improved)	3	95	3	52
Impulse Rose	5	68	5	62
Super Elfin Red	5	86	5	42
Super Elfin Coral	3	98	3	25
Super Elfin Pink	3	96	3	26
		$LSD_{0.05}$=4		

APPENDIX B: PROTEIN AND LIPID CONTENTS OF IMPATIENS SEEDS

Procedure - protein determination

One-hundred milligrams of oven-dried impatiens seeds were ground in a mortar. One gram of sea sand was ground with the seed to absorb the oil. The nitrogen content of the sample was determined by using a semimicro-kjeldahl method (Bremner and Breitenbeck, 1983). The percentage protein content was estimated by multiplying the nitrogen content (mg) per 100 mg impatiens seed by 6.25.

Results

Impatiens seed percent protein content

Cultivar	No. Samples	Protein (%)
Super Elfin Orchid	7	26.5 ±0.6
Accent Orange	5	24.1 ±0.6

Recovery tests: 96%

Lipid determination

One-hundred milligrams of oven dried impatiens seeds were ground in a mortar. One gram of sea sand was ground with the seed to absorb the oil. The lipid content was estimated by using a modification of the procedure described by Lehle et al., 1983. The ground sea sand-impatiens mixture was distilled in a microsoxhlet apparatus with 10 ml isopropyl alcohol for 2 hours. The isopropyl alcohol was removed by drying at 47C under a nitrogen gas stream. The result of 11 determinations on Super Elfin Orchid seeds was 51% lipid (sd=3).

APPENDIX C: TESTS ON THE INHIBITION OF IMPATIENS SEED GERMINATION

Exogenous Ethanol

Impatiens seeds were imbibed in solutions that contained various concentrations of ethanol either on blotter paper or submerged in 40 ml of the solution. Germination counts were taken after seven days.

Ethanol (%)	Blotter (B) or Submerged (S)	Germination (%) after 5 days
0.0	B	93
0.0	S	100
0.001	B	91
0.01	B	93
0.05	B	81
0.1	B	44
0.5	B	0
1.0	B	0
1.0	S	0
5.0	B	0
5.0	S	0

Potassium Cyanide

Ten millimolar potassium cyanide completely inhibited the germination of 'Super Elfin Orchid' seeds on blotter paper or submerged in water.

Vacuum Evacuated Water

Forty 'Super Elfin Lipstick' impatiens seeds were submerged in 40 ml water. Oxygen was evacuated from the system for approximately 5 minutes. No germination ocurred in 7 days and 83% of the seeds had germinated 7 days after the seeds were transferred to blotter paper.

Summary

Increased concentrations of exogenous ethanol were needed to block germination of impatiens seeds on blotter paper. Lower concentrations of ethanol probably are metabolized by the seeds.

Both cyanide and evacuated water inhibited germination of impatiens seeds, and this indicated that oxidative phosphorylation and oxygen are necessary, at least to a small extent, for germination to occur.